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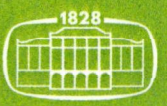

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Acta Agronomica Hungarica, 52(1), pp. 1–8 (2004)

DEVELOPMENT OF DIPLOID POLLEN IN SPIKELET CULTURES OF BARLEY (*HORDEUM VULGARE* L.) AND RYE (*SECALE CEREALE* L.)

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Received: 12 January, 2004; accepted: 11 March, 2004

Colchicine is a plant alkaloid, known for thousands of years and currently used widely for the doubling of the genome in plant and animal cells due to its antimitotic effect.

The aim of the present experiments was to develop stable autodiploid pollen grains *in vitro* in diploid lines of rye (*Secale cereale* L.) and barley (*Hordeum vulgare* L.) and to use these in intra- and interspecific crosses. Spikelet cultures of one rye and one barley variety were subjected to colchicine treatment in different stages of development and under differing *in vitro* conditions. Exposure to colchicine led to a drastic reduction both in the number of fertile pollen grains and in the percentage seed-setting, which was only observed in cultures inoculated in the early binuclear microspore stage. On medium containing colchicine the seed-setting percentage was 1.6% for barley and 0.1% for rye. Flow cytometry and root tip analysis revealed that all the progeny barley plants were diploid, while in the case of rye one was tetraploid, indicating that the egg cell may also be diploidised by colchicine treatment.

Key words: spikelet culture, colchicine, diploid pollen, *Hordeum vulgare* L., *Secale cereale* L.

Introduction

One of the major problems facing plant breeders is how to transfer favourable traits (e.g. cold and drought tolerance, disease resistance) from one cultivated species to another without causing a deterioration in their agronomic properties. One possibility is the development of interspecific hybrids. Difficulties may be encountered during the sexual crossing of different plant species due to abnormal chromosome pairing during meiosis, with the result that most of the hybrids are partially or completely sterile. Fertile progeny can only be obtained if the genome is reduplicated in the hybrid and normal meiosis takes place. Since the likelihood of spontaneous reduplication is extremely small, colchicine treatment is applied in order to create artificial interspecific hybrids. The traditional technique involves dipping the seedlings obtained by crossing into colchicine solution, but this has the disadvantage that a large proportion die, while those that survive are almost exclusively chimeric.

If diploid gametes could be produced and fused, the problem of chromosome pairing could be avoided. Although unreduced pollen grains suitable for the development of polyploids do occur in nature (Sala et al., 1989; Mashkina et al., 1998; Park et al., 2002), their occurrence is extremely species-

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specific and rare, so in most cases they must be produced artificially (Carputo et al., 1997). One way of doing this is to rediploidise the male gametes using colchicine.

Colchicine, the drug contained in *Colchicum* species, causes the disintegration of the nuclear spindle threads, thus preventing the separation of the chromatides or chromosomes during cell division and inducing endomitosis, i.e. the doubling of the genome within the cell (Salmon et al., 1984). During the several thousands of years of its history this drug was used both as a medicine and as a poison. Since its rediscovery in recent times its antimitotic effect has been widely exploited for the creation of polyploid individuals in both the animal and plant kingdoms (Eigisti and Dustin, 1955). All parts of the plant organism have been subjected to colchicine treatment in order to observe its effect on the structure of the chromosomes and the cell nucleus. In the thirties and forties of the 20th century the development of polyploid plants using colchicine treatment was an extremely fashionable field, and it was during this period that the development of diploid and polyploid gametes was first reported. Colchicine treatment on microspores at the cytological level has varying consequences depending on the stage of development. Microspore mother cells go through "c-meiosis", resulting in the production of diploid or polyploid monads (Walker, 1938). If uninuclear microspores are treated with colchicine, distinctive "c-mitosis" is obtained (Levan, 1939; Dermen, 1940). This leads to the development of uninuclear diploid microspores, which follow the normal path of differentiation to produce diploid pollen grains, depending on the colchicine concentration and the length of the treatment.

Successful pollen maturing experiments were reported more than thirty years ago in flower cultures of the species *Cucumis* and *Nicotiana* (Porath and Galun, 1967; Hicks and Sussex, 1970). Tanaka and Ito (1980) produced functional pollen grains, capable of developing normal pollen tubes, by isolating uninuclear microspores in the G1 phase of the cell cycle in lilies and tulips. In the following years mature flowers of numerous dicotyledonous species were successfully produced in *in vitro* cultures from flower primordia (Rastogi and Sawhney, 1989), but only in very few species did the isolated immature flowers, anthers or microspores develop normally and produce fertile pollen. Very little work was done on cereal species, though considerable progress was achieved in gametophyte cultures in maize (Pareddy and Petolino, 1992) and wheat (Trionne and Stockwell, 1989). In these experimental systems, although pollen fertility and seed setting were substantially lower than the values achieved *in situ*, they nevertheless reached a level of almost 50%.

In recent years, an efficient *in vitro* flower culturing technique has been elaborated in the Martonvásár laboratory, allowing both microspore mitosis events to take place in several *Poaceae* species (Barnabás and Kovács, 1992) and leading to the development of diploid pollen grains in spikelet cultures of *Triticum turgidum* ssp. *carthlicum* (Takács et al., 1999). It thus appears that this method will enable diploid pollen grains to be developed in a number of cereal species.

This paper reports on the effect of colchicine treatment in rye and barley spikelet cultures on microsporogenesis and on the ploidy levels of the progeny.

Materials and methods

In vitro spikelet cultures were initiated from one barley variety (Igri) and one rye variety (Lovászpatonai). After 56 days of vernalisation at 2°C plants of these varieties were grown in phytotron chambers (Conviron GB-48) with a constant day/night temperature of 15/17°C and a 12/12 h photoperiod with a light intensity of 200 μmol/m²/s. Spikes were sterilised in 20% household bleach for 20 min and rinsed in distilled water. Before removing spikelets for inoculation on nutrient medium, anther samples were taken from each spike and carmine acetic acid staining was used to check the developmental stage of the microspores. Spikelets containing microspores in the late uninuclear or binuclear stage were used for culturing. The spikelets were removed from the flower axis with a scalpel and placed on modified MS (A4) medium containing 0.02% or 0.04% colchicine (Barnabás and Kovács, 1992). The control medium did not contain the antimitogenic agent. Sucrose was used in the medium as carbon source. The pH of the medium was adjusted to 5.8 or 4.5. After 1–6 days of colchicine treatment the spikelets were transferred to colchicine-free medium and were kept in a tissue culture chamber at 20°C with 16/8 h illumination at a light intensity of 25 μmol/m²/s until full maturity (approx. 8 weeks).

As rye is an open-pollinated species, the pollination of the spikelets must be carried out artificially: 15–20 days after inoculation the mature yellow anthers protruded from the spikelets and the feathery styles also became visible. After a short period of drying the anthers dehisced and the pollen was used to pollinate spikelets from other spikes. The embryos were excised from the endosperm-deficient grains and placed on hormone-free B5 medium.

The ploidy level of the seedlings was determined by flow cytometry. A leaf section measuring approx. 1 cm² was taken from each seedling and placed in 800 μl Nuclear Buffer I (Galbraith et al., 1983). The cell nuclei were liberated by "chopping" with a razor blade and filtering through a 30 μm nylon filter. After 10 minutes of incubation 500 μl of the cell nucleus suspension was stained with 6.5 μl propidium iodide (PI) at a concentration of 1 mg/ml. Measurements were made using a Becton Dickinson FACScan flow cytometer and the data were analysed using CellQuest software.

Chromosome preparations were made after fixing the root tips of the germinated embryos in Carnoy solution (1:3 mixture of absolute ethanol and conc. acetic acid) using the method described by Molnár-Láng and Sutka (1994), and were stained with the traditional Feulgen method.

For the purpose of light microscope observations, anthers of pollen samples from each pollen-maturing variant were fixed in Carnoy solution and stained with carmine acetic acid.

For the analysis of pollen tube growth, anthers which ripened *in vitro* were removed from the spikelets when still moist and left to dry in a sterile box before being used for pollination. The pistils used in the experiment were taken from the diploid plants raised in growth chambers as spikelet donors. In the control experiments pollination was carried out using haploid pollen taken from intact plants. The pollinated pistils were kept in a humid environment at room temperature for three hours, after which they were stained with cotton blue (D'Souza, 1972).

The microscope studies were carried out using an Olympus BX 51 light microscope.

Results and discussion

Approximately two weeks after the spikelet cultures were initiated, most of the spikelets turned brown when the pH of the nutrient medium was 5.8. In these spikelets the development of both the anthers and the pistils ceased and they died off. On pH 4.5 medium the spikelets remained green until the anthers had ripened and only then started to turn brown. Cytological analysis of the pollen developing in *in vitro* cultures demonstrated that practically all the

microspores were destroyed on pH 5.8 medium, irrespective of whether the spikelets were inoculated in the late uninuclear or early binuclear microspore stage. On pH 4.5 medium the microspores only remained viable in spikelets which were cultured in the early binuclear microspore stage and were not treated with colchicine for more than 3 days. The data clearly prove that *in vitro* culture in itself causes a great drop in pollen quality (Takács, 1994), while in combination with colchicine it leads to a drastic reduction in the quantity of fertile pollen produced (Takács et al., 1999). The vast majority of the surviving microspores developed into trinuclear pollen grains and exhibited great similarity to pollen maturing *in vivo*. Some of these pollen grains exhibited diploid character (pollen size larger than the control; shorter, wider male gametes) (Fig. 1). In the case of rye, colchicine treatment also caused characteristic morphological changes in the structure of the microspores. In addition to the typical longish pollen grains, round or aberrant forms were also found (Fig. 2).

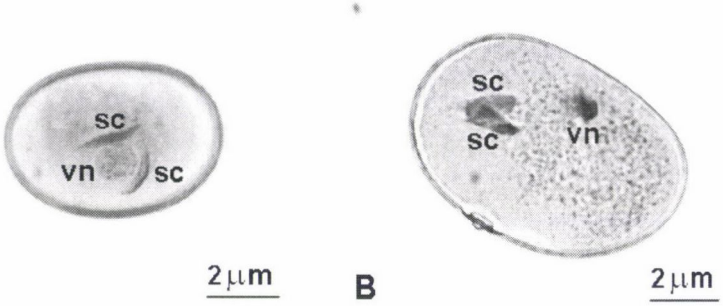

Fig. 1. Haploid (A) and diploid (B) rye pollen grains. vn = vegetative cell nucleus; sc = sperm cell

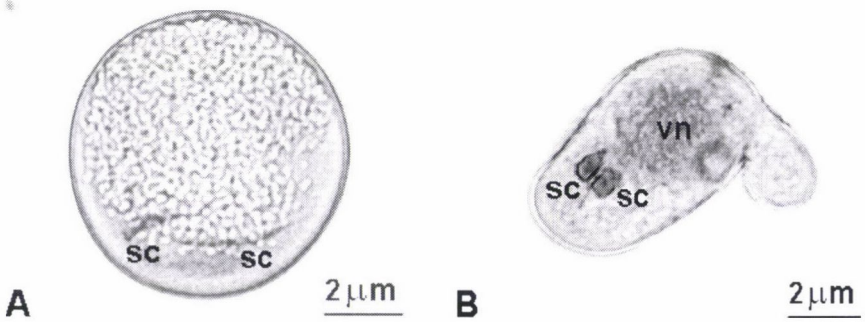

Fig. 2. Morphological changes in rye pollen as the result of colchicine treatment. A: round, B: aberrant pollen grains. vn = vegetative cell nucleus; sc = sperm cell

In order to determine whether pollen grains developed *in vitro* were capable of fertilisation, intraspecific crosses were made: 1. As a control experiment, castrated flowers of 2n plants were pollinated with 1n pollen from plants grown in the growth chamber, 2. Castrated flowers of 2n plants were pollinated with *in vitro* 1n pollen from spikelet cultures without colchicine treatment. Taking the seed set values obtained in the control experiment as 100%, seed settings of 72% and 58% were obtained in the 2nd experiment for rye and barley, respectively. As could be concluded from the cytological analysis, the addition of the drug caused a substantial reduction in the seed setting in *in vitro* spikelet cultures. At a pH of 5.8 seed setting was only obtained in the barley variety Igri, and then only on control medium. A pH of 4.5 proved more favourable for seed setting. A total of 39 seeds were obtained from 1680 cultured barley spikelets and 8 seeds from 3360 rye spikelets (on control and colchicine-containing medium), equivalent to a fertilisation rate of 2.3% and 0.24%, respectively. The seed setting percentage on medium containing colchicine was found to be 1.6% for barley and 0.15% for rye. The extent to which the fertility of the cultures depends on the colchicine concentration is not completely clear. Although better results were obtained with a concentration of 0.02% as regards both pollen quality and seed setting, the differences were not significant. In the case of seed setting the values are so low that statistical analysis is completely pointless. According to Belea (1986) treatment at low concentration for a longer period gives better results than a short period of exposure to higher concentrations. This suggests that it would be advisable to apply a concentration of 0.01%, perhaps for a longer period.

All the seed set were endosperm-deficient (Fig. 3). The embryos excised from them and transferred to hormone-free medium all germinated and developed into healthy seedlings (Fig. 4).

When the ploidy levels of the seedlings were checked using a flow cytometer, one tetraploid (4n = 28) rye plant was found, which originated from a spikelet culture treated with 0.02% colchicine (Fig. 5). This result was confirmed by chromosome counting on the root tip (Fig. 6).

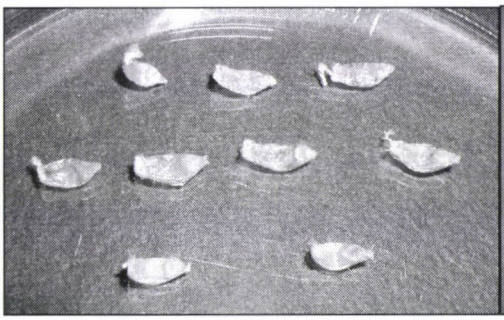

Fig. 3. Endosperm-deficient barley seeds

Fig. 4. Barley embryos excised from endosperm-deficient seeds and germinating on hormone-free medium

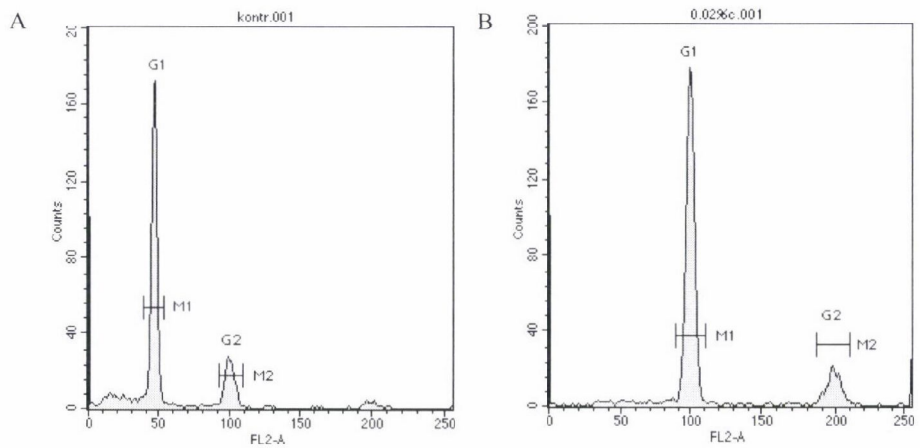

Fig. 5. Results of flow cytometer measurements on rye seedlings. A: Control (diploid), B: Produced on nutrient medium containing 0.02% colchicine. Cell nuclei isolated from tetraploid leaves contain twice as much DNA as the control, as indicated by the shift in the G1 peak from channel 50 to channel 100

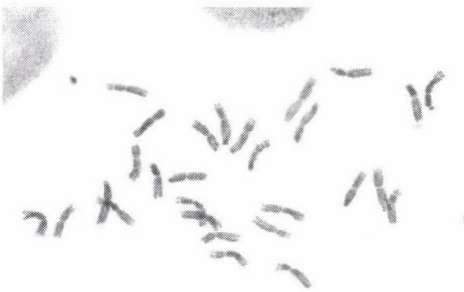

Fig. 6. Chromosomes in the metaphase of the tetraploid rye plant (4n=28)

The course of development for the embryo sac and the microspore under *in vivo* and *in vitro* conditions was studied by Tímár et al. (1997) in various species of wheat. It was found that the embryo sac became fully developed 2–3 days before the appearance of mature, trinuclear pollen grains. In experiments carried out on *Triticum turgidum* ssp. *carthlicum* by Takács et al. (1999) all the seeds that developed were triploid, probably due to the fusion of haploid egg-cells and diploid male gametes. It would appear that, due to this 2–3-day difference in maturity, the egg-cell in spikelets containing microspores in the early binuclear stage is sufficiently mature to be immune to the doubling effect of the colchicine in the culture medium. Although the species examined by these authors did not include either *Hordeum vulgare* or *Secale cereale*, it is unlikely that these two species differ to any great extent from the various wheat species

in this respect. Nevertheless, in the present experiments triploid seeds were not formed in either species. The single tetraploid rye seed probably arose when a diploid male gamete fertilised a diploid egg-cell. It appears unlikely that a haploid egg-cell fused with a triploid pollen grain. The data from the literature cited above suggest that the diploidisation of the egg-cell occurs rarely under the conditions used for the spikelet culture, but, as proved by the development of the tetraploid rye seed, is not impossible. It is conceivable that fusions between diploid pollen and haploid egg-cells also took place, but the zygotes or embryos of barley or rye may not have been viable under the given culture conditions.

As all the seeds set in barley originated from haploid pollen, it could be that haploid and diploid pollen grains have different chances of forming pollen tubes on diploid styles. As seen in Fig. 7, the control (haploid) pollen grains developed significantly longer tubes in given time than those originating from *in vitro* cultures, thus being at an advantage in the fertilisation race.

In summary, it can be stated that the colchicine treatment of spikelet cultures could be a suitable method for the development of diploid gametes of barley and rye, but if they are to be used for crossing related species, making new gene sources available to breeders, the method needs to be further improved.

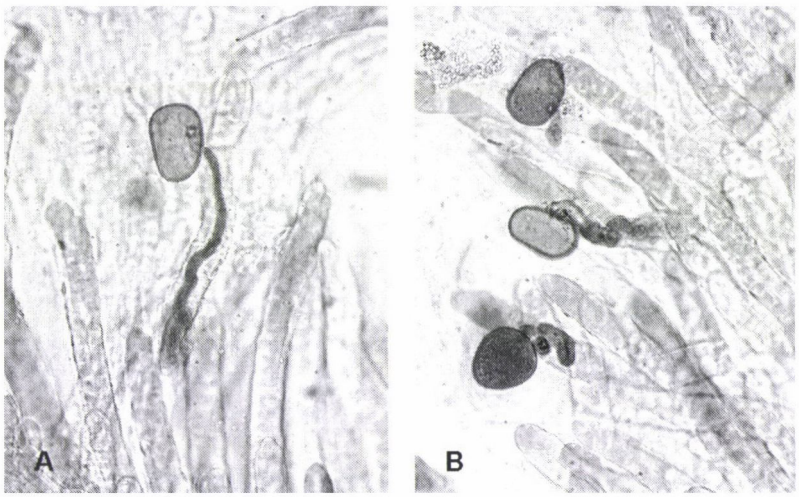

Fig. 7. Pollen tube growth of haploid (A) and diploid (B) rye pollen on diploid styles

Acknowledgements

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NUTRIENT STATUS AND ENZYME ACTIVITY ALTERATION IN CUCUMBER SEEDLINGS AS A RESPONSE TO BORON DEFICIENCY

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Cucumber (*Cucumus sativus* L. var. Beit alpha) seedlings were grown in two groups on boron-deficient (traces of boron) and boron-sufficient (10.0 μM boron) hydroponic media for 30 days under controlled conditions. At harvest, the concentrations of magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were determined in addition to boron (B) in the dry tissues of roots and leaves. The concentration of phenolic compounds in the roots was also determined. Peroxidase (POD) and catalase (CAT) enzyme activity was assayed in the fresh plant material. In addition, changes in the peroxidase and catalase isozyme patterns were also identified. The results showed that the vegetative growth of cucumber plants was negatively affected by boron deficiency. Biomass accumulation decreased by as much as 24.3% in the shoots and 49.1% in the roots. The nutrient concentrations in both the leaves and roots of B-stressed plants were substantially lower. Phenolic compounds were accumulated in significant amounts in the roots of deficient plants. The peroxidase and catalase enzyme activities were significantly increased in the tissues of deficient plants and new isozymes were induced or activated. The irregular biochemical changes occurring in B-deficient plants were explained as a plant physiological response to B-deficient conditions.

Key words: boron deficiency, ion contents, peroxidase, catalase, cucumber

Introduction

The essential importance of boron for higher plants was established early in the 20th century by Warington (1923). Recent studies, however, showed that most boron is localized in the cell wall, especially in the case of boron deficiency (Hu and Brown, 1994; Matoh et al., 1996). Boron deficiency was found to affect nutrient concentrations, uptake and balance in the plant tissues. As to its effect on the plasma membrane, Cakmak et al. (1995) suggested that the primary effects of boron deficiency would be an increase in membrane permeability, leading to nutrient leakage from the cell membrane. Muehling et al. (1998) observed that the quantity of bound cellular calcium was low in boron-deficient faba bean plants. In addition, the nitrate content in tobacco leaves was reported to dramatically decrease in boron-deficient plants (Camacho-Cristobal and Gonzalez-Fontes, 1999). On the other hand, Zude et al. (1997) found that the foliar application of boron increased the concentrations of calcium, potassium and magnesium in apple leaves.

Boron was reported to play a key role in carbohydrate transport (Lewis, 1980) and its metabolism by controlling the amylases, reductases and dehydrogenases in plant tissues (Goldbach, 1997). It was also hypothesized that boron stimulates IAA-oxidase and would thus reduce the auxin level to the limit which allows the subsequent growth of the roots (Jarvis et al., 1984). Boron-deficient plants were found to accumulate polyphenolic compounds through the oxidation of phenols and the formation of free radicals (Cakmak et al., 1995). An increase in the peroxidase (Mittler et al., 2001) and catalase (Willekens et al., 1997) activities was reported as an early response to stress to provide resistance against the formation of free radicals.

The present work aimed at studying the status of certain nutrients and the related enzyme activity in cucumber seedlings as a response to boron deficiency in hydroponic growth medium.

Materials and methods

Plant material and growth conditions

Seeds of cucumber (*Cucumus sativus* L. var. Beit alpha) were germinated on filter paper moistened with 0.2 mM $CaSO_4$. After 5 days incubation at 24°C in the dark the seedlings were transferred into 2.0 l aerated plastic pots filled with full strength nutrient solution of the following composition: 0.7 mM K_2SO_4; 0.1 mM KCl; 2.0 mM $Ca(NO_3)_2$; 2.0 mM $MgSO_4$; 0.1 mM KH_2PO_4; 0.5 µM $MnSO_4$; 0.5 µM $ZnSO_4$; 0.2 µM $CuSO_4$; 80 µM Fe EDTA and 0.01 µM $(NH_4)_6Mo_7O_{24}$ with or without the addition of 10.0 µM boron (B) in the form of boric acid. The experiment was carried out in three replicates.

The pH of the nutrient solution was adjusted to 6.2 using NaOH and the whole culture nutrient solution was renewed every three days.

The plants were maintained for 30 days under controlled environmental conditions (light/dark regimes of 16/8 h, temperature 24/20C°, relative humidity 65%, light intensity 200 µM $m^{-2}s^{-1}$). Each measurement was done in triplicate.

Sampling and sample preparation

On the 30th day of growth, the cucumber plants were harvested. The plant samples were divided into roots and shoots, washed with bidistilled water and oven dried at 65°C for 24 hours, then weighed and ground. At the same time, fresh samples were taken to assay phenolic compounds and enzyme activity.

Analytical methods

Elements

A 1 g sample was dry-ashed in a muffle furnace at 550 °C for 6 hours using 3.0 N HNO_3. The residue was then suspended in 0.3 N HCl.

Magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were measured using an atomic absorption spectrophotometer (Zeiss PMQ3). Boron was extracted according to Wimmer and Goldbach (1999) and measured using a UV-VIS-spectrophotometer (Perkin-Elmer Lambda 2).

Phenolic compounds

As phenolic compounds are mostly accumulated in the roots, total phenols were extracted from root tissues according to Swain and Hillis (1959) using 80% methanol, and detected according to Lam and Street (1971). The blue colour developed was measured at 725 nm using a UV-VIS spectrophotometer.

Enzyme activity

Extraction procedure

Extraction was carried out according to Polar (1976). The plant tissues were excised and homogenized in 250 mM sucrose, 0.2 mM DTT, 2% polyvinyl polypyrrolidine (w/v) and 0.1 mM EDTA. The pH of the grinding buffer was adjusted to 7.2 and the homogenate was filtered through four layers of cheesecloth and centrifuged at 12,000 r.p.m. for 20 min.

Enzyme assay

Peroxidase (POD) activity was determined in the supernatant according to Amako et al. (1994). The reaction mixture consisted of 1.5 ml of 100 mM K-phosphate buffer (pH 6.8), 1.0 ml of 60 mM pyrogallol; 0.48 ml of 0.6 mM H_2O_2 (30%) and 20 µl of the crude enzyme extract. The increase in absorbance at 430 nm was recorded using a UV-VIS spectrophotometer.

Catalase (CAT) activity was assayed at 240 nm according to Chance and Maehly (1955) in a total volume of 1.0 ml of 25.0 mM K-phosphate buffer (pH 6.8), 10 mM H_2O_2 (30%) and a diluted enzyme extract.

Isozyme electrophoresis

Non-denaturing polyacrylamide gel electrophoresis (PAGE) was carried out according to Davis (1964) using 7.5% polyacrylamide using vertical apparatus (Hofer SE600,14×16×0.75 cm). Staining was carried out as follows:

For peroxidase (POD), the gels were stained with O-dianisidine as described by Amako et al. (1994). The appearance of dark brown bands was caused by the peroxidase activity of the respective isozymes in the gel.

Catalase (CAT) isozymes were detected according to the method of Woodbury et al. (1971). The gel was soaked in 5 mM phosphate buffer (pH 7.0), then transferred into 5.0 ml 30% H_2O_2. After 10 min the gel was washed with water and stained with a reaction mixture containing 2% (w/v) ferric chloride and 2% (w/v) K-ferricyanide. The enzyme appeared as yellow bands on a dark green background. The reaction was then stopped with water and the gel was photographed.

Data analysis

The data were statistically analysed using the Costate Statistical Package (Anonymous, 1989).

Results

Dry mass accumulation

The dry mass accumulation in the untreated plant shoots and roots was found to be 24.3% and 49.1% lower, respectively, compared to that of boron-sufficient plants (Fig. 1).

Tissue nutrient concentrations

Boron-deficient plants were found to contain lower Mg, Fe, Mn and Zn concentrations than control plants (Table 1). It was clear that Mn was accumulated in the roots of boron-deficient plants. The copper concentration in the roots was the same in the two treatments, but its translocation to the leaves appeared to be negatively affected in boron-deficient plants.

Phenols and enzyme activity

Significant amounts of phenolic compounds were found to accumulate in the roots of boron-deficient cucumber plants (Fig. 2). The specific activities of peroxidase (POD) and catalase (CAT) in the leaves and roots of boron-deficient plants were significantly higher than in the boron-sufficient plants (Table 2). The highest activity of both POD and CAT was found in the roots of boron-deficient plants.

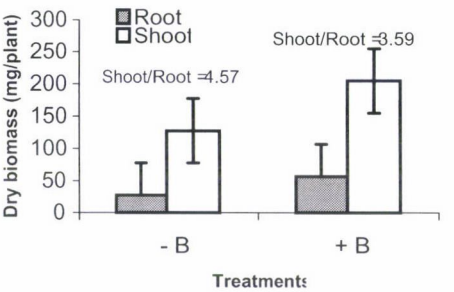

Fig. 1. Dry biomass accumulation in the roots and shoots of 30-day-old cucumber plants as affected by the boron level in the growth medium

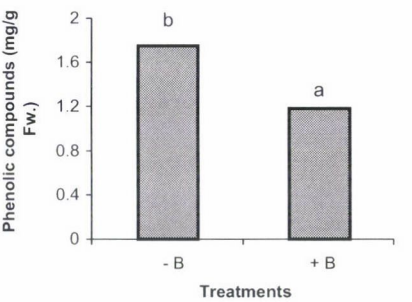

Fig. 2. Quantity of phenolic compounds (C_6H_5OH equiv. mg/g Fw.) in 30-day-old cucumber roots as affected by the boron level in the growth medium

Table 1

Boron and other nutrient concentrations in the leaves and roots of 30-day-old cucumber plants as affected by the boron level in the growth medium (dry weight basis)

Treatment	B (ppm)		Mg %		Fe (ppm)		Mn (ppm)		Zn (ppm)		Cu (ppm)	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
+B	48b	55b	1.04b	1.01a	110b	175b	31.8a	92.5b	22.5b	23.0b	5.0a	10.0a
–B	13a	16a	0.75a	0.81a	70.0a	75.0a	60.0b	23.0a	12.0a	14.0a	5.0a	6.0a
$LSD_{5\%}$	8.01	12.5	0.16	N.S.	17.92	61.03	19.2	5.77	6.25	8.01	N.S.	N.S.

Columns with the same letters are not significantly different; N.S. = non-significant

Table 2

Specific peroxidase (POD) and catalase (CAT) activity in the roots and leaves of 30-day-old cucumber plants as affected by the boron level in the growth medium

Treatment	Peroxidase activity (EU/mg protein/min)		Catalase activity (μM H_2O_2 consumed/mg protein/min)	
	Root	Leaf	Root	Leaf
+B	272.7a	118.55a	311.4a	98.9a
–B	688.4b	145.64b	477.1b	221.3b
$LSD_{5\%}$	63.8	11.33	90.96	12.62

Columns with the same letters are not significantly different

Isozyme expression

Peroxidase (POD)

The expression of POD was detected in the water-soluble protein fraction of the roots and leaves of cucumber plants (Fig. 3). Electrophoretic analysis showed that four distinct isozyme patterns were exhibited in the roots of boron-deficient plants (lane 1), whereas in the roots of boron-sufficient plants (lane 3) only two isozymes with different intensities were observed. Four bands with different Rf values were exhibited in boron-deficient leaves (lane 2), while two bands with different mobility were observed in the leaf tissue of plants grown at sufficiency level (lane 4). It was obvious that the root POD isozyme activity increased under boron deficiency, which was in line with the results obtained for POD activity, as presented in Table 2.

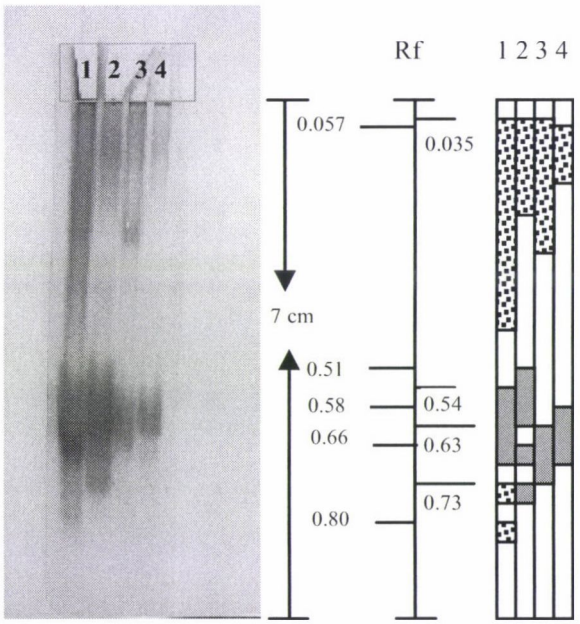

Fig. 3. Isozyme banding patterns of peroxidase (POD) in 30-day-old cucumber roots and leaves as affected by the boron level in the growth medium

Lane 1 = Boron-deficient root
Lane 2 = Boron-deficient leaf
Lane 3 = Boron-sufficient root
Lane 4 = Boron-sufficient leaf

Dark Faint

Catalase (CAT)

The CAT isozyme patterns are shown in Fig. 4. Only one band was exhibited by the roots and leaves of B-deficient and B-sufficient plants. Boron-deficient roots showed a faint band with slow mobility (Rf =0.18) (lane 1), while the roots of boron-sufficient plants also showed one band with high intensity (lane 3), but a different Rf value (0.21). Meanwhile, one band with much higher intensity was observed in boron-deficient and boron-sufficient leaves (lanes 2 and 4, respectively), having different Rf values (0.08 and 0.06).

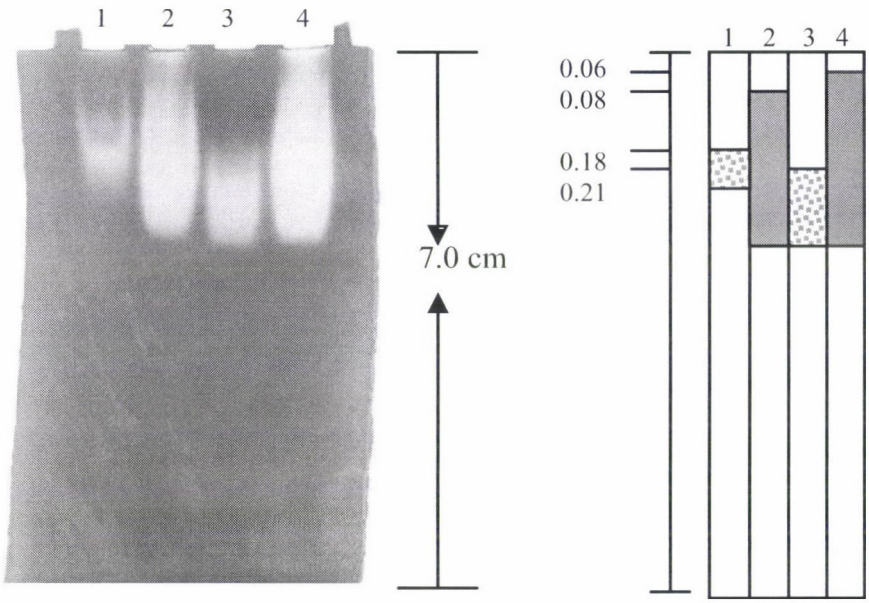

Fig. 4. Isozyme banding patterns of catalase (CAT) in 30-day-old cucumber roots and leaves as affected by boron levels in the growth medium

Lane 1 = Boron-deficient root
Lane 2 = Boron-deficient leaf
Lane 3 = Boron-sufficient root
Lane 4 = Boron-sufficient leaf

Dark Faint

Discussion

Boron was found to play a role in carbohydrate transport (Lewis, 1980) and metabolism (Goldbach, 1997). Thus, boron deficiency was interpreted as a reduction in dry biomass accumulation. On the other hand, the lower shoot/root ratio of B-deficient plants illustrates that the roots are more severely affected than the shoots. Similar findings were found by Dell and Huang (1997) and Camacho-Cristobal and Gonzalez-Fontes (1999) for other plants.

The boron shortage in the culture medium of untreated cucumber plants led to its deficiency in both roots and leaves. The lower concentrations determined for other nutrients, i.e. Mg, Fe, Zn and Cu (but not Mn) were reflections of the boron insufficiency in the root cell membrane, causing a disturbance in membrane permeability for these nutrients and creating nutrient imbalance within the plant tissues. Cakmak et al. (1995) even found nutrient leakage from sunflower tissues in the case of severe boron deficiency.

Boron deficiency was also postulated to aggravate oxidation reactions in plant tissues (Marschner, 1995). Thus, the increase in the concentration of phenols in the roots of B-deficient plants proved that the roots were suffering from boron deficiency, which led to the oxidation of phenolic compounds.

The increase in the H_2O_2-detoxifying enzyme activity in the plant tissues was another phenomenon found to correlate with boron deficiency. Peroxidase activity was 2.5-fold higher in the roots but only slightly increased in the leaves, while catalase activity was 1.5-fold higher in the roots and 2.2-fold higher in the leaves of B-deficient cucumber plants compared to B-sufficient ones. The lack of boron may cause higher sensitivity to light, enhancing the synthesis of additional amounts of peroxidase and catalase in the roots and leaves of B-deficient plants. The high activity of these enzymes can be explained as a plant physiological defence mechanism aimed at scavenging excess amounts of free radicals which may be generated during photorespiration (Conklin et al., 1996).

Various isoperoxidases were formed in the root and leaf cells of B-deficient cucumber plants. Four distinct patterns with different intensities, having Rf values of 0.035, 0.54, 0.73 and 0.80, were present in the cells of deficient roots, while only two patterns with the same intensity and Rf values of 0.035 and 0.63 were present in the root cells of B-sufficient plants. In B-deficient leaf cells, there were again four distinct patterns differing in intensity with Rf values of 0.035, 0.51, 0.66 and 0.73, versus two patterns with different intensity and Rf values of 0.057 and 0.58 in the leaf cells of B-sufficient plants. The catalase patterns were completely different in number, intensity and mobility in B-deficient roots and leaves compared with B-sufficient plants. A larger number of patterns with higher intensity were found in B-deficient plants. This means that the induction or activation of different isozymes at the gene level took place as the plants responded to boron deficiency. This may also explain the additional amounts of peroxidase and catalase synthesized to defend the plants against the harmful effects of excess hydrogen peroxide. Similar results were reported by Palavan-Unsal et al. (2002).

In conclusion, boron deficiency caused a reduction in dry mass accumulation in the shoots and roots of cucumber plants. Due to the free radicals formed as a result of low boron levels in the plant tissues, phenolic compounds are accumulated in the roots. As the plants responded to boron deficiency, the peroxidase and catalase enzyme activities in the root and leaf tissues increased and the induction or activation of both peroxidase and catalase isozymes took place.

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LOW TEMPERATURE EFFECTS ON THE GROWTH OF EVENING PRIMROSE (*OENOTHERA* SPP.) ROSETTES

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In field crops of evening primrose (*Oenothera* spp.) the post-winter growth of rosettes is slow to re-start. The effect of temperature on the growth of rosettes was assessed in a controlled environment experiment. Relative growth rate was positively correlated with temperature, but in apparent contrast to the results from field trials, the rosettes grew at constant temperatures as low as 6.5°C. However, following transfer to warmer temperatures an increase in relative growth rate did not occur until 7–10 days later, whilst a change to a cooler environment caused an immediate reduction in relative growth rate. Thus, it seems likely that growth is inhibited by intermittent exposure to temperatures of 0°C or below. Partitioning of biomass between root and shoot was independent of temperature, but at 6.5°C the relative rate of leaf area increase was very low. Consequently, the specific leaf area was lower in rosettes growing at lower temperatures.

Key words: evening primrose, *Oenothera* spp., rosette growth, biomass, low temperatures, growth cabinets

Introduction

Evening primrose (*Oenothera* spp.) has become established as a high-value agricultural crop and has been successfully grown in north and central Europe, North America and New Zealand (Simpson and Fieldsend, 1993). Its value is derived from its seed oil, which contains γ-linolenic acid, an essential fatty acid with recognised nutritional and pharmaceutical properties (Horrobin, 1990). It can be grown as either an overwintered or a spring-sown crop.

Fieldsend and Morison (2000) reported that the growth of overwintered evening primrose rosettes was very slow to re-start post-winter, but in 1997 growth was recorded approximately 50 days earlier than in 1996 (Figs. 1a and 1b). The major environmental difference between the two years was temperature. In 1996, the daily mean temperature remained almost continuously below 5°C until day of year 97, whereas in 1997 the daily mean temperature exceeded 5°C from day 37 onwards. Similarly, the daily maximum temperature continuously exceeded 10°C from day 97 in 1996 and day 50 in 1997, and in the latter year minimum temperatures fell below 0°C on only one day after this date. Day 97 in 1996 and day 50 in 1997 corresponded closely with the start of crop growth. Results consistent with this pattern were subsequently obtained from a similar field trial in 1998 (Fieldsend and Morison, 1999) (Fig. 1c).

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In early 1998 a controlled environment experiment was set up to assess the effect of low temperatures on the growth of evening primrose rosettes. The choice of minimum temperature was constrained by the specification of the growth cabinets. To investigate why no growth was recorded in the 1996 crop between days 40 and 97 when, apart from one day, temperatures did not exceed 10°C (but rarely fell below 5°C) and when the mean daily maximum temperature was 7.1°C, rosette growth was evaluated at 7°C, 10°C and two higher temperatures, 13°C and 16°C.

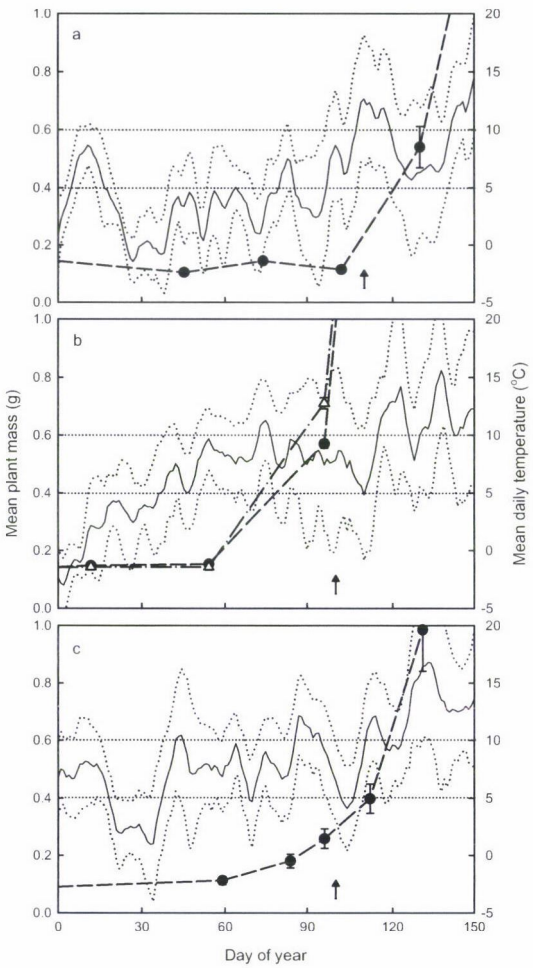

Fig. 1. Time course in the field in mean overwintered plant mass above and below ground (g) in (a) 1996, (b) 1997 and (c) 1998 in cv. Merlin (closed circles) and cv. Peter (triangles). Arrows indicate approximate date of onset of stem extension and error bars represent ± 1 SE. Also shown is mean (solid lines) and maximum and minimum daily (dotted lines) temperatures (five-day moving averages). Adapted from Fieldsend and Morison (2000) (a, b) and Fieldsend and Morison (1999) (c)

Materials and methods

Plant material

Seeds of evening primrose cv. Merlin stock S1416 were sown into a peat-based potting compost (Levington M2, Fison plc) in a seed tray on 24 September 1997 and the tray was placed in an incubator at 25°C to assist germination. On 6 October 120 seedlings were pricked out into modules (P28, Plantpak Ltd., Maldon, UK), with one seedling per module, in a glasshouse with natural light and heating to provide frost protection. The plants were potted on into a mixture of potting compost, sand and soil from the Terling trial site in 75 mm diameter pots on 22 November and were moved outside on 7 January 1998. On 1 March, the rosettes were potted on into the same potting compost, sand and soil mix in 0.94 litre pots and were transferred to the growth cabinets the following day.

The controlled environment

On 2 March, defined as day 1, the plants were placed in two growth cabinets (model 600H, Gallenkamp, Loughborough, UK) set at 7.3°C (compared with a mean outside temperature of 7.4°C for the preceding 15 days) to acclimate. Throughout the experiment, 12 hour days were used (compared with a natural daylength of 11 h) with approximately 300 μmol m^{-2} s^{-1} PAR being provided by fluorescent tubes supplemented by incandescent fluorescent bulbs. On 11 March twenty plants were harvested for growth analysis and the rest were divided equally between four growth cabinets set at four temperatures, nominally 7, 10, 13 and 16°C. In practice, temperatures varied slightly from these figures and actual temperatures were recorded using a data logger (21x, Campbell Scientific Ltd., Shepshed, UK) and thermocouples recording at one minute intervals and averaged over 30 minutes. Following each subsequent harvest, the plants were rearranged within the cabinets to minimise the effects of any spatial variation in the environment in the cabinets.

Experimental methods

For the initial growth analysis 20 plants were used, but assessments were carried out on ten plants from each treatment at each subsequent harvest (Table 1). Following the separation of shoot and root the number of leaves longer than 5 mm on each plant was counted and (with the exception of the initial harvest) the projected area of the rosette was measured by placing the intact shoot upside down on a leaf area meter (Mk 2, Delta-T Devices Ltd, Cambridge, UK). The roots were separated from the soil and were carefully washed to recover as much of the fibrous roots as possible. All harvested material was then dried at 80°C in a forced-draught oven and weighed with an analytical balance.

Table 1
Summary of harvest dates and treatment changes

Date	Day number	Harvest	Comments
Wed 11 March	10	initial	Plants then placed in 7°C, 10°C, 13°C and 16°C
Fri 20 / Sat 21 March	20	first	
Tue 31 March	30	second	Some plants then exchanged between temperatures
Wed 8 April	38	third	
Mon 27 April	57	final	plants at 16°C and 7°C (ex-16°C) only
Tue 28 April	58	final	plants at all other temperatures

Results

Rosette growth to day 30

Harvests were carried out at day 20 and day 30 and progressively more rapid growth took place with increasing temperature (Fig. 2). An increase in plant mass and number of leaves longer than 5 mm per plant was recorded at all temperatures including the lowest, nominally 7°C but in fact 6.5°C. However, at 6.5°C very little increase in leaf area occurred.

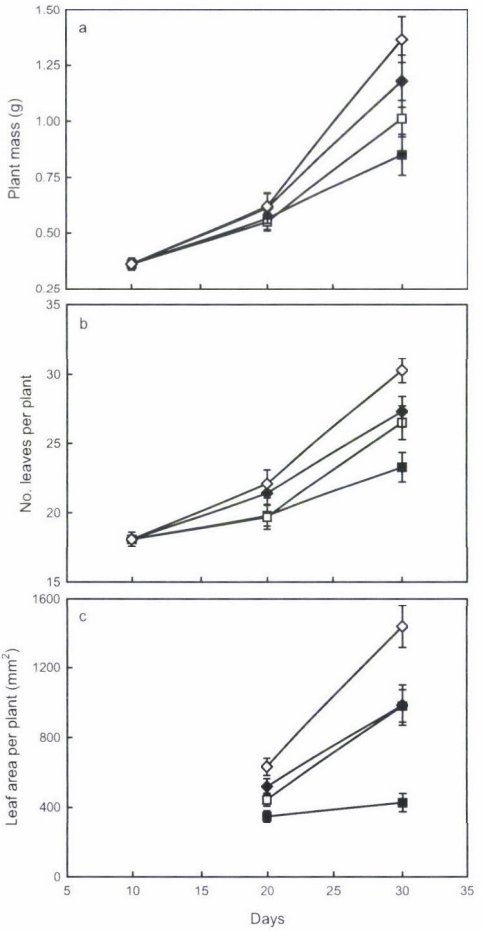

Fig. 2. Time course to day 30 in evening primrose cv. Merlin in (a) mean overwintered plant mass above and below ground (g), (b) mean number of leaves per plant and (c) mean leaf area per plant (mm^2) in growth cabinets at 7°C (closed squares), 10°C (open squares), 13°C (closed diamonds) and 16°C (open diamonds). Error bars represent ± 1 SE

Rosette growth from day 30

Since by day 30 it was evident that evening primrose rosettes were growing at near-constant temperatures as low as 6.5°C, it was decided to investigate the effect of change of temperature on growth. On day 30 the plants growing at 7°C were exchanged with those growing at 13°C and subsequent harvests of plants growing at 7, 10 and 13°C were carried out on days 38 and 58. Half of the plants growing at 16°C were also transferred to the 7°C growth cabinet on day 30 and these were harvested on day 57, together with the plants which remained at 16°C.

By day 57 plants which remained at 16°C were larger with respect to mass, leaf number and leaf area than those transferred from 16°C to 7°C (Fig. 3); indeed no increase in leaf area was recorded in the plants moved to the cooler temperature. Similarly, by day 58 the plants transferred from 7°C to 13°C were equal to, or larger than, those transferred from 13°C to 7°C. After day 30 the leaf area of plants moved from 7°C to 13°C increased by 138% in eight days whilst little increase in leaf area occurred between days 30 and 58 in the plants moved from 13°C to 7°C.

Partitioning, specific leaf area and relative growth rate

Assimilate partitioning was not affected by temperature (Fig. 4a). As the rosettes grew, the root to shoot weight ratio remained almost constant up to a total plant weight of approximately 4 g, the partitioning coefficient, i.e. the slope of the linear regression fitted to these data (Wheeler et al., 1994) being 0.96. Root weight increased proportionately more than shoot weight in larger rosettes but it is unclear whether this was a symptom of the plants becoming pot-bound (and hence allocating more resources to root growth in response to reduced nutrient availability) or a natural consequence of rosette development.

Specific leaf area declined as the rosettes grew larger (Fig. 4b) but following day 30 a 45% increase in specific leaf area occurred in the plants which had been transferred from 7°C to 13°C. On day 57 the specific leaf area of the plants moved from 16°C to 7°C was 15% smaller than that of the plants which remained at 16°C.

If relative growth rate (RGR, g g^{-1} day^{-1}) between days 20 and 30 is plotted against temperature, the data fall close to a straight line which can be extrapolated through the origin, suggesting that the base temperature for the growth of evening primrose rosettes is near 0°C (Fig. 5a). However, with the exception of the plants growing at 7°C values of RGR for the period between days 10 and 20 fell below the line with, for example, the RGR of rosettes growing at 16°C being only 60% of the expected value. These values were close to that which would have been expected prior to day 10 when all rosettes were growing at a temperature of 7.3°C. On day 38 the values of RGR of plants growing at 7°C (ex-13°C) and 10°C fell close to the line, but that of plants growing at 13°C (ex-7°C) was low. By contrast, on day 58 the RGR of the plants

growing at 7°C and 13°C were close to the expected values. The RGR of rosettes growing at 10°C was 50% higher than expected, which might be explained by very high root weights (>3 g) for four of the ten plants. On day 57 the RGR of the rosettes growing at 16°C was unchanged from day 38 but the RGR of the plants growing at 7°C (ex-16°C) was approximately 30% lower.

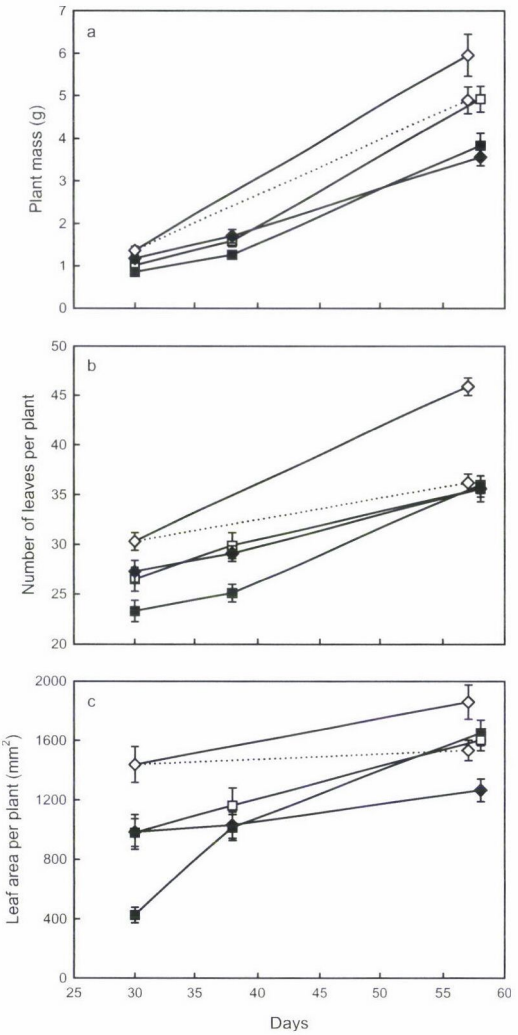

Fig. 3. Time course from day 30 in evening primrose cv. Merlin in (a) mean overwintered plant mass above and below ground (g), (b) mean number of leaves per plant and (c) mean leaf area per plant (mm^2) in growth cabinets at 13°C (ex-7°C) (closed squares), 10°C (open squares), 7°C (ex-13°C) (closed diamonds), 16°C (open diamonds) and 7°C (ex-16°C) (open diamonds, dotted line). Error bars represent ± 1 SE

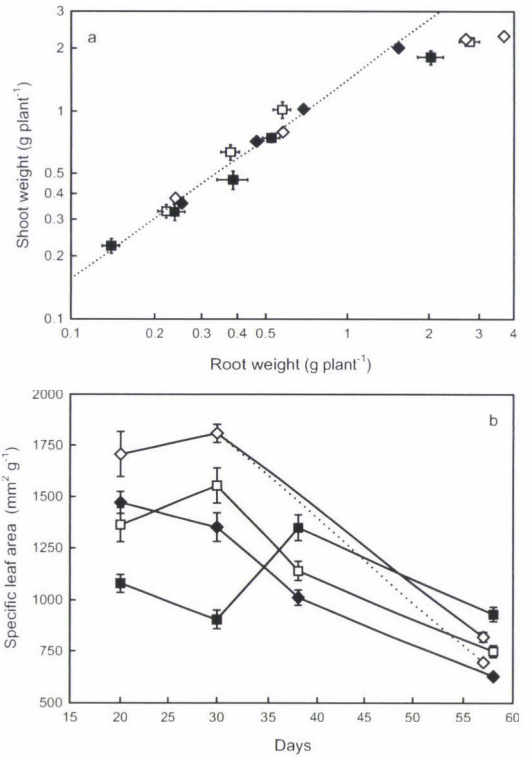

Fig. 4. (a) Relationship in evening primrose cv. Merlin between mean shoot mass (g) and mean root mass (g) of plants initially allocated to growth cabinets at 7°C (closed squares), 10°C (open squares), 13°C (closed diamonds) and 16°C (open diamonds). Plant weight is shown on a logarithmic scale. The linear regression is of the form $y = 0.96x + 0.15$. (b) Time course in mean specific leaf area ($mm^2\ g^{-1}$) of plants initially allocated to growth cabinets at 7°C (closed squares), 10°C (open squares), 13°C (closed diamonds) and 16°C (open diamonds). The dotted line denotes plants transferred to 7°C on day 30. The arrow indicates when some plants were transferred to different temperatures. Error bars represent ± 1 SE

A different relationship existed between relative rate of leaf area increase (RLI, $mm^2\ mm^{-2}\ day^{-1}$) and temperature (Fig. 5b). For three treatments the values for RLI between days 20 and 30 fell close to a straight line but the RLI for the plants growing at 10°C was anomalously high. This latter value should be treated with reserve, as on day 22 it was reported that the tungsten bulbs in this growth cabinet had occasionally remained on all night and at this time the rosettes in this treatment had distinctly more erect leaves. The lighting problem was rectified on day 22, the leaf angle differences subsequently disappeared and the RLI of these plants between days 30 and 38 was relatively low. Also between days 30 and 38, following the exchange of plants between cabinets, the

RLI of plants growing at 13°C (ex-7°C) was extremely high (0.17 mm^2 mm^{-2} d^{-1}) but that of plants growing at 7°C (ex-13°C) was very low. Similarly, on day 57 the RLI of plants growing at 7°C (ex-16°C) was very low, whilst a low RLI value was also obtained for the plants growing at 16°C, which may reflect the increased partitioning of assimilate into roots in these large rosettes. On day 58 the RLI of plants growing at 7°C (ex-13°C) remained very low, that of plants growing at 10°C was close to the expected value and that of plants growing at 13°C (ex-7°C) was lower than expected. A line fitted by eye to the data suggests that the base temperature for leaf expansion in evening primrose rosettes is approximately 6°C.

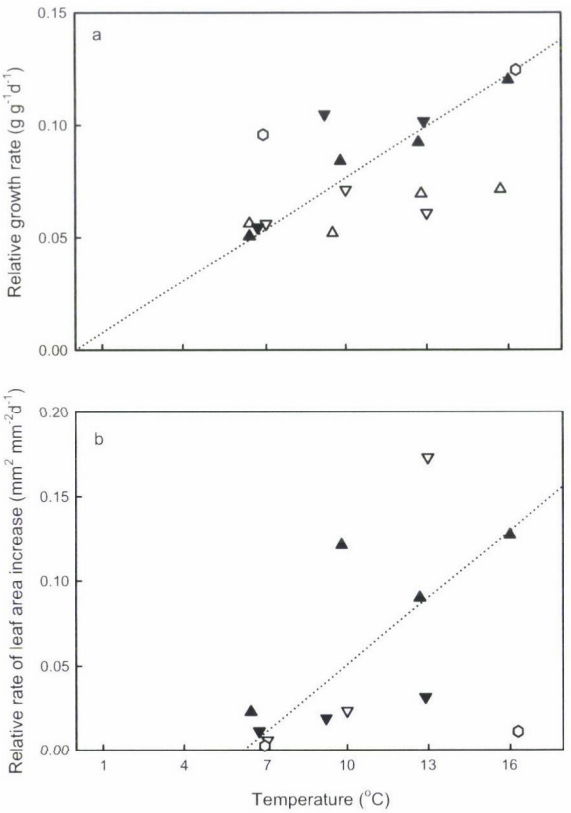

Fig 5. (a) Mean relative growth rates (g g^{-1} d^{-1}) and (b) mean relative rates of leaf area increase (mm^2 mm^{-2} d^{-1}) in evening primrose cv. Merlin plants in growth cabinets from day 10–20 (open triangle up), day 20-30 (closed triangle up), day 30-38 (open triangle down), day 38-58 (closed triangle down) and day 30–57 (hexagon). The functions are of the form y = 0.008x (a) and y = 0.013x –0.080 (b)

Discussion

In apparent contrast to the results from field trials reported by Fieldsend and Morison (1999; 2000), this controlled-environment study has shown that evening primrose rosettes can grow (i.e. increase mass) at temperatures as low as 6.5°C and that the base temperature for growth may be 0°C. However, with the temperatures used in this study an increase in RGR did not occur until approximately 7–10 days after the onset of higher temperatures (Fig. 5a) and there was some evidence that a change to a cooler environment (e.g. from 13°C to 7°C) caused an immediate reduction in RGR to the rate appropriate to the lower temperature. The possibility that the growth of evening primrose rosettes is inhibited by intermittent exposure to temperatures of 0°C or below is broadly consistent with the results from the field trials. In 1996 minimum temperatures frequently fell below 0°C until day 97 and no crop growth was recorded until after day 102 (Fig. 1a). In 1997 and 1998 minimum temperatures were consistently higher than 0°C after day 36 and growth commenced after day 54 in 1997 and day 59 in 1998 (Figs. 1b and 1c). The slower subsequent increase in plant size in 1998 compared to 1997 may be attributable to brief periods around days 47 and 70 when the minimum temperature fell below 1°C.

Despite an increase in biomass (Figs. 2a and 3a) and a constant partitioning coefficient (Fig. 4a), there was very little increase in the projected leaf area of evening primrose rosettes growing at lower temperatures (Figs. 2c and 3c) and consequently the specific leaf area in these plants was low (Fig. 4b). The increase in the number of leaves longer than 5 mm per plant at temperatures of 6.5–7°C (Figs. 2b and 3b) shows that some leaf growth was in fact taking place and that some assimilate was being incorporated into structural tissue. Reekie and Reekie (1991) observed that in evening primrose rosettes growing at temperatures ranging between 15 and 30°C the petioles of the lower leaves elongated as the rosettes became larger and hence self-shading within the rosette did not increase. Petiole growth did not occur in this study at temperatures of 6.5–7°C.

Although the effect of low temperature on the RGR of evening primrose rosettes can be attributed to a change in the photosynthesis : respiration balance, the precise mechanism is not clear from this study. The accumulation of soluble carbohydrates at low temperatures inhibits net photosynthesis via a feedback mechanism (e.g. Hurry et al., 1998). However, even though photosynthetic rates were likely to have been limited by temperature Mendham et al. (1981) observed that growth in winter oilseed rape could be "appreciable" before the "beginning of spring" (defined as the date when mean temperatures rose consistently above 5°C). By contrast, in 1996–98 no "appreciable" growth occurred in evening primrose rosettes before the "beginning of spring", which coincided closely with the end of minimum temperatures of 0°C or below.

The slow post-winter start to growth leads to late leaf canopy closure and makes evening primrose crops very susceptible to weed competition. A quicker resumption of growth following periods of low temperatures may be an effective selection criterion for breeding new evening primrose cultivars for use as overwintered crops.

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INFLUENCE OF WATER STRESS ON SPRING BARLEY YIELDS UNDER POLISH CLIMATIC CONDITIONS

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The aim of this study was to evaluate the influence of atmospheric precipitation on the yield of spring malting barley. The plant height and heading of the studied forms were observed as additional indicators of their reaction to variable water conditions. The plant material for this study consisted of spring barley breeding lines in generations F_6–F_7 evaluated at 7 locations in 1996–2001. The highest yield was observed with precipitation within the range 258-321 or 356–382 mm per growing season in years with colder or warm weather, respectively. These results were obtained using abundant plant material highly differentiated genetically, so it may be inferred that the above values are the rainfall levels optimal for spring barley cultivation under Polish climatic conditions.

The experimental locations could be divided into four classes according to observations on mean yields and on total rainfall before heading and between heading and full maturity. The optimal class included locations where the highest yield was observed; in the second there was a high precipitation level but a lower yield was obtained; in the third class there was a shortage of rainfall before heading, and in the fourth class there was a shortage of rainfall between heading and maturity. The observation of yields lower than those obtained in optimal locations led to the assumption that stress factors at these locations did not allow the yield potential of the studied genotypes to be fully expressed. The studied genotypes showed good adaptation to the variable conditions of the Polish climate, which is characterized by periods with a shortage or excess of rainfall.

Key words: water stress, spring barley, yield

Introduction

An adequate water supply is the fundamental condition for the proper course of all life processes in plants, so it is obvious that it is the major factor influencing the yield of cereals. Water shortages are the main problem facing agriculture in Mediterranean and tropical countries. However, gradual changes in the environment have caused the appearance of this problem in countries in the temperate zone, where it did not have any practical importance until recently (Eckersten et al., 2001; Foulkes et al., 2001; Slavik and Zavadil, 2001). In the abundant literature on this topic there is hardly any information on barley, due to the common belief that this species is resistant to unfavourable environmental conditions, including water deficits (Ceccarelli, 1996). However, the vegetation period of spring barley is close to 100 days and both shortages and excess of water are disadvantageous for it (Gąsiorowski, 1997; Pecio, 2002) and may significantly decrease the yield. So there arises the need to introduce new cultivars giving high, stable yields not only under optimal climatic conditions

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but also under unfavourable conditions. The analysis of yields in variable conditions may thus be useful in breeding as well as in determining which regions new cultivars should be grown in. The aim of this study was to evaluate the influence of the amount of atmospheric precipitation on the yield of spring malting barley. The height and heading of the plants were also observed as additional measures of their reaction to changing water availability.

Materials and methods

The plant material in this study consisted of breeding lines of spring barley in generations F_6–F_7 evaluated in field trials in Plant Breeding Stations in Bąków, Modzurów, Łagiewniki, Polanowice, Radzików, Nagradowice and Strzelce in 1996–2001. The set of studied genotypes varied over the years of the study; however, in each year the same forms were studied in all locations. In total about 700 genotypes were studied. In all locations the plants were grown on 10 m^2 plots with the standard level of fertilization in a randomized block design with four replications. Two-way variance analysis was carried out, where the mean squares were calculated for the interactions between the analysed traits.

The rainfall levels were recorded in all locations from sowing to harvest. The number of days between 1 May and heading was counted during the vegetation period. The plant height was measured before harvest and the seed yield was calculated at 15% seed moisture and expressed in q/ha.

The locations in which the highest yield was recorded in the given year were regarded as optimal. The mean yields from these locations were the basis for calculations of the stress intensity and the index of genotype stress susceptibility. The indexes were calculated according to the formula given by Fischer and Maurer (1978) and commonly used in the study of drought resistance (Kłodnicka 1999; Gautam et al. 2000):

$$S = \frac{1 - Y_s / Y_0}{D}$$

where:

S – stress susceptibility index ;

Y_S – the mean yield of the genotype under stress conditions;

Y_0 – the mean yield of the same genotype under optimal conditions;

D – stress intensity

$$D = \overline{Y}_s / \overline{Y}_0$$

$\overline{Y}_s$ – the mean yield of all the genotypes under stress conditions

$\overline{Y}_0$ – the mean yield of all the genotypes under optimal conditions

Phenotypic correlation coefficients were computed using values of the investigated traits averaged across years and precipitation levels: optimal, high, deficit before heading date and deficit between heading and maturity.

Results and discussion

Each year the locations in which the field trials were conducted were ranked according to decreasing rainfall totals over the whole vegetation period (Table 1). The comparison of rainfall levels and the mean values of the analysed traits revealed an interesting regularity: the highest yield in a given year was correlated with a certain level of precipitation (figures in bold in Table 1).

Table 1
Total precipitation in seasons and mean values of observed traits in 1996–2001

Years	Locations	Mean rainfall per season (mm)	Yield q/ha	Plant height (cm)	No. of days from 1 May to heading
1996	Modzurów	402.8	44.94	72.54	56.98
	Bąków	395.3	59.31	70.04	44.55
	Łagiewniki	**321.4**	**73.62**	**75.32**	**50.47**
	Radzików	315.8	48.17	65.40	57.95
	Nagradowice	272.4	66.61	74.32	43.54
	Polanowice	241.4	53.83	78.49	46.80
	Strzelce	237.1	68.88	76.05	44.94
1997	Bąków	552.9	53.36	70.07	48.58
	Modzurów	552.4	62.46	75.32	47.03
	Polanowice	466.2	55.97	90.45	46.80
	Strzelce	295.1	71.20	99.69	48.70
	Łagiewniki	**280.5**	**75.36**	**80.06**	**49.76**
	Radzików	226.3	55.70	86.57	53.77
	Nagradowice	203.5	60.15	86.90	46.10
1998	Polanowice	362.3	41.29	70.90	39.94
	Bąków	333.2	36.11	56.08	37.47
	Radzików	289.1	53.73	70.10	50.73
	Modzurów	282.7	59.22	68.06	40.47
	Strzelce	**258.5**	**77.33**	**86.25**	**39.32**
	Łagiewniki	200.5	56.82	65.90	42.00
	Nagradowice	189.6	74.68	73.92	36.88
1999	Bąków	367.5	48.19	66.35	37.33
	Modzurów	338.8	57.85	73.18	41.52
	Radzików	325.0	45.20	65.60	44.51
	Polanowice	316.1	53.56	88.77	36.63
	Strzelce	295.5	58.03	87.40	38.67
	Nagradowice	**283.7**	**65.67**	**90.59**	**43.75**
2000	Bąków	493.6	56.31	70.63	36.30
	Polanowice	433.5	60.20	68.59	39.49
	Modzurów	**382.9**	**77.40**	**72.33**	**39.33**
	Nagradowice	287.3	48.38	61.11	34.85
	Łagiewniki	280.0	52.65	58.06	34.15
	Radzików	180.3	48.03	54.38	38.84
2001	Bąków	486.5	53.90	76.19	52.47
	Polanowice	451.3	41.67	70.82	48.30
	Modzurów	387.0	51.41	75.32	50.47
	Łagiewniki	**356.0**	**75.16**	**84.46**	**51.80**
	Radzików	342.7	62.43	78.45	50.64
	Strzelce	297.6	68.07	98.81	49.65
	Nagradowice	261.4	54.42	67.67	46.28

The mean yields obtained in locations with either higher or lower rainfalls were significantly lower.

Table 2
Analysis of variance for studied traits in 1996–2001

Source of variability	Mean square for interaction			
	Yield × height	Yield × heading	Heading × height	Total
Locations	15061.49**	5267.955**	7389.694**	10411.14**
Genotypes	238038**	86755.85**	612204.2**	312332.7**
Interaction	8217.857**	5326.332**	7146.378**	6896.856**
Error	33.18888	15.88686	21.66669	23.58081

The plant heights and days between May 1 and heading did not show such a regularity. The analysis of variance (Table 2) revealed that the differences observed in the performance of the breeding lines between locations and years were significant. Significant interactions were found between the studied genotypes and the environment as well as between the studied traits. The maximum yield was observed at precipitation levels between 258 and 321 mm per growing season in 1996–1999. However, the optimal amount of rainfall was 356–382 mm/season in 2000–2001. This increase in the water demand was most probably caused by the exceptionally high temperatures observed in these years and the loss of water due to the increased transpiration rate (Machado and Paulsen, 2001). The results were obtained from observations on a wide, genetically diverse plant material (about 700 genotypes) studied in different locations over a long period, so the optimal rainfall levels for spring barley grown under Polish climatic conditions could be determined.

The values shown in Table 1 were indirectly supported by the study of Shakhatreh et al. (2001), carried out in a warm climate with a rainfall level of 150–350 mm/season, where the level of 350 mm was found to be sufficient. The observed decrease in the mean yield due to both a shortage or excess of precipitation seems to confirm Gąsiorowski's (1997) suggestions of the harmful influence of these environmental factors. Similarly, Pecio (2002) found that low precipitation and a temperature in the range of 6–8°C was needed for the optimal growth of malting barley (especially in the first stages, immediately after sowing). It has been known for many years, and confirmed by more recent studies, that the effects of water deficiency depend largely on the plant growth phase in which the stress occurs (Sullivan and Eastin, 1974; Kamińska and Mazgalska, 1992; Abayomi and Wright, 1999; Yang et al., 2000). For this reason, and also due to the fact that plant heading was evaluated, the growing season was divided into two parts: until heading and until full maturity. The analysis of mean yields with regard to the total rainfall in each of these parts of the growing season allowed the experimental locations to be ranked into four classes: optimal, giving the highest yield in the given year; with a high precipitation level but a lower mean yield; with a shortage of rainfall before heading; and with a shortage of rainfall between heading and maturity.

The observation of a lower than optimal yield in a given location suggested that a stress factor occurred which did not allow the yield potential of the studied genotypes to be fully expressed. The intensity of the stress (D) and the stress susceptibility index of the studied genotypes were calculated for these locations. The calculations were performed only for the yield, since this trait is expressed as the result of processes taking place within the plant and is influenced by environmental stress. The results obtained for locations with a high level of precipitation in both periods of plant growth are shown in Table 3 and those for locations with a deficiency of rainfall in Table 4.

A comparison of the data presented in Tables 3 and 4 shows that the intensity of both forms of water stress (high and low rainfall levels) were rather high. The stress susceptibility index (S) ranged from 0.03 to 0.64 in "dry" locations (Table 4) and from 0.05 to 1.13 in "wet" habitats (Table 3). It seems that the studied lines were more resistant to water shortage than to high rainfall levels. These results confirm the disadvantageous influence of excessive rainfalls on spring barley cultivation.

The yield was significantly correlated with the plant heights in all the studied locations (Table 5). However, optimal conditions short forms of barley gave higher yields, whereas under both types of water stress tall forms performed better. A significant correlation between the number of days before heading and the yield was only observed in habitats with rainfall deficits and was independent of when the drought occurred.

Table 3

Stress intensity (D) and mean stress susceptibility index (S) in locations with a high level of precipitation in 1996–2001

Years	Locations	Total precipitation (mm)		Stress intensity (D)	Mean stress susceptibility index (S)
		Before heading	Before maturity		
1996	Modzurów	114.2	288.6	0.61	0.64
	Bąków	222.8	172.5	0.80	0.24
	Radzików	143.5	172.3	0.53	0.65
1997	Bąków	146.9	406.0	0.71	0.41
	Modzurów	114.1	438.3	0.83	0.20
	Polanowice	181.5	284.7	0.74	0.34
	Strzelce	138.5	156.6	0.94	0.05
1998	Bąków	124.8	208.4	0.47	1.13
	Polanowice	197.9	164.4	0.53	0.88
	Radzików	180.6	108.5	0.69	0.44
1999	Bąków	167.5	200.0	0.75	0.32
	Modzurów	182.8	156.0	0.88	0.13
	Polanowice	119.1	197.0	0.83	0.20
	Strzelce	179.9	115.6	0.88	0.12
2000	Bąków	261.9	231.7	0.73	0.37
	Polanowice	211.9	221.6	0.78	0.28
2001	Bąków	272.8	213.7	0.72	0.39
	Modzurów	105.6	281.4	0.68	0.46
	Polanowice	333.7	117.6	0.55	0.81

A similar phenomenon was observed by Shakhatreh et al. (2001) who found that the relationship between the observed traits depended upon the environment. This shows the necessity of carrying out breeding and selection programmes with special regard to the environmental conditions under which the future cultivars will grow.

The higher yields given by late forms of barley (Tables 1 and 5) suggest that in the case of rainfall deficit before heading there is some chance that the plants will "outgrow" the unfavourable period. The significant correlation between the yield and the number of days before heading, observed even in the case of water shortage after heading, seems to imply the existence of a genotypic relationship between these traits. However, additional studies are needed for an explanation of this problem, the results of which will be described elsewhere.

Table 4
Stress intensity (D) and mean stress susceptibility index (S) in locations with rainfall deficits in 1996–2001

Years	Locations	Total precipitation (mm)		Stress intensity	Mean stress susceptibility index (S)
		Before heading	Before maturity		
		Deficit of precipitation before heading			
1996	Strzelce	82.9	154.2	0.94	0.06
1998	Modzurów	73.5	209.2	0.77	0.30
2000	Nagradowice	98.9	188.4	0.62	0.62
2000	Radzików	67.3	113	0.61	0.64
		Deficit of precipitation between heading and maturity			
1996	Polanowice	182.8	58.6	0.73	0.37
1997	Radzików	154.9	71.4	0.93	0.28
	Nagradowice	118.1	85.4	0.80	0.25
1998	Łagiewniki	132.2	68.3	0.73	0.36
	Nagradowice	143.8	45.8	0.97	0.03
1999	Radzików	289.3	35.7	0.70	0.43
2000	Łagiewniki	196.1	83.9	0.68	0.47
2001	Nagradowice	205.7	55.7	0.72	0.38

Table 5
Correlations between the studied traits in different habitats

Precipitation	Traits	Yield	Height
Optimal	Height	–0.579*	
	Heading	–0.128	–0.035
High level	Height	0.642*	
	Heading	–0.003	0.030
Deficit before heading	Height	0.959**	
	Heading	0.912**	0.758**
Deficit between heading and maturity	Height	0.311	
	Heading	0.702**	-0.303

Conclusions

1. The highest yield of spring barley was observed with precipitation in the ranges of 258–321 and 356–382 mm per season in cold and warm years, respectively. These results were obtained using a large, genetically differentiated plant material, so it could be concluded that the above values are the optimal rainfall levels for this crop under Polish climatic conditions.
2. Most of the spring malting barley genotypes studied showed low susceptibility to the stress caused by an excess or shortage of precipitation, confirming their usefulness for cultivation under variable climatic conditions.
3. Short forms of spring barley gave higher yields under optimal conditions, whereas tall genotypes yielded better under stress conditions caused by an excess or shortage of water.
4. A significant correlation between the number of days before heading and the yield was only observed in habitats with rainfall shortages. This correlation did not depend upon the period when the water shortage occurred, and late forms gave higher yields.
5. The results obtained demonstrate the necessity of considering the environmental conditions under which the cultivars will be grown in the course of barley breeding and selection.

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AN ECOLOGICAL LOOK AT SALINE WETLANDS

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Due to the occurrence of considerable areas of wetlands in the world, the wise, sustainable use of these lands is one of major importance for ecologists and agriculturists. As the presence of indicator species and plant communities can be a measure of the compatibility between plants and edaphic conditions in these regions, the ecological niches of plant species in part of the southern coastal areas of the Caspian Sea have been studied to show the correlation of each species with its own habitat. The plant communities were separated with Ward's cluster analysis. The correlation of these communities and plant species with environmental factors was investigated with the CCA method, using PC-Ordination-4 software. The results showed that the soil EC, water table, soil pH, SAR and ESP were 14–157 dS/m, 0–240 cm, 6.5–8.5, 13.4–84.8 and 2–55%, respectively. This range of values, in addition to creating ecological niches for species with different ecological roles, was also effective in the formation of plant communities. The analysis of vegetation and soil data with the CCA method showed the relationships between soil factors and vegetation. In spite of the dominance of the species *Halocnemum strobilaceum* in all the plant communities, the correlation of this species with plant species such as *Aeluropus littoralis, Salicornia europaea, Aeluropus lagopoides, Salsola aurantia* and *Puccinella distans* in relation to changes in EC, water table, pH, SAR and ESP, is important from the point of view of sustaining the physical environment and ecological function. The simplification of these ecosystems (by drainage, agriculture, etc.) may disturb the natural equilibrium. As these ecosystems are susceptible and changes in their use are costly from the ecological and economic points of view, the wise use of ecosystems in their natural forms (rangelands and habitats) is recommended to prevent the spread of salinity and to protect habitats and biodiversity.

Key words: saline wetlands, salinity, Iran, ecology, ecological niches, *Halocnemum strobilaceum*

Introduction

"Salinity" is one the basic factors that reduces plant growth and yield in the world. Almost 10% of the 7000 million ha arable lands of the world consist of saline and sodic soils (Tanji, 1990). As the presence of any plant is an indication of the special compatibility mechanism of the plant in the area under investigation, the recognition of the native and resistant species in saline soils, the introduction of established plant communities in these conditions to restore the vegetation, and the demonstration of the relationship of soil and water salinity with plants, could be a useful approach to the wise management of these regions.

The area under investigation is part of the saline areas in the southern coastal lands of the Caspian Sea. This region is called the "the axis of Iranian agriculture" due to its favourable climate and fertile lands. In this part of the

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world, the salinization of the lands (resulting from the unsuitable use of soil and water) is one of the problems facing ecologists and agriculturists. Frey and Probst (1986), Akhani and Ghorbanli (1993) and Jafari (1994) carried out studies on saline lands in Iran. Ayoub and Malcolm (1993) and Malcolm and Choukr-Allah (1995) studied the Daghestan lands in Russia and found the species *Halocnemum strobilaceum* to be one of the most resistant plant species to salinity, sodicity and soil water-logging. Alakhverdiev (1988) investigated the reasons for the distribution of the species *Aeluropus littoralis,* which occupies lands with less salinity in comparison to *Halocnemum strobilaceum.* In his opinion, the presence of *Aeluropus littoralis* along with *Halocnemum strobilaceum* shows an improvement in the soil conditions with respect to salinity and sodicity.

Other than ecologists, botanists, agriculturists (Kernick, 1986) and researchers (Smith et al., 1981) also studied these lands from the range management point of view and classified the forage plants of saline lands based on their resistance to salinity.

Since the area of saline soils is increasing worldwide due to high technology, changes in agricultural practices and an understanding of the guidelines provided by nature will be required for the wise use and control of saline lands. The recognition of the ecological niches of existing species in these ecosystems is important for this purpose.

Materials and methods

The saline southern Caspian Sea wetlands include arable lands, plain rangelands and the habitats of migratory birds. In this research, carried out on part of the southern Caspian Sea coastal lands, the saline lands were specified based on a Land Form Map using data obtained from satellite pictures of the series "LANDSATS" with a scale of 1:500.000.

The area of the region is 1772.5 km^2, the general slope is less than 0.5%, the local topography intensity is less than 1 m, and the soils are hydromorphic and sedimentary halomorphic. The typical soils of these lands are located around the Gorgan Bay area on the borderline of Iran and Turkmenistan. The mean annual rainfall is 350 mm and the elevation from mean sea level is –20 to +20 m. Study sites were selected on the southeastern coast of the Caspian Sea and plant and soil evaluations were made on ecological units. The Flora of the USSR (Komarov, 1968–1980) and the Flora Iranica (Rechinger, 1963–1996) were used to identify the plants. By setting transects 100 m long and selecting every 5 m as a random point, the relative vegetation canopy was evaluated using the Nearest Individual method (Holechek et al., 1995). To study the ecological niches of the plant species, the water table in each ecological unit was measured in observation wells and the soils were sampled from 0–30 cm depth to determine EC, pH, SAR and ESP. The plant communities were separated by Ward cluster analysis (Ward, 1963) and the correlation of these communities and plant species with environmental factors was investigated by the CCA method (Ter Braak, 1987) using PC-Ordination 4 software.

Results

The following species were identified in these ecosystems:

Halocnemum strobilaceum, Aeluropus littoralis, Aeluropus lagopoides, Puccinella distans, P. maritima, Frankenia hirsuta, Limonium gemelini, Halostachys caspica, Salicornia europaea, Aster tripolium, Psylostachys spicata, Lycium turkemanicum, Spergularia rubra, Capparis spinosa, Ceratocapus arenarius, Kochia arenaria, Noea macronata, Tamarix galica, T. ramosissima, Salsola aurantia, S. crassa, S. dendroides, S. kali, Hordeum glaucum, Typha angustifolia, Juncus maritima, Cressa cretica, Artemisia herba-alba, Lophocloa phleoides, Parapholis incurva, Suaeda maritima, Seidlitzia florida, Alhagi camelorum, Trifolium fragiferum, T. tomentosum, Plantago cornopus, Plantago maritima, Heliotropium europaaea, Sagina annua and *Zingeria trichopoda.*

The results of Ward cluster analysis classified the vegetation of the region into 5 communities (Fig. 1):

- *Halocnemum strobilaceum* + *Aeluropus littoralis*
- *Halocnemum strobilaceum* + *Aeluropus lagopoides*
- *Halocnemum strobilaceum* + *Salicornia europaea*
- *Halocnemum strobilaceum* + *Puccinella distans*
- *Halocnemum strobilaceum* + *Salsola aurantia*

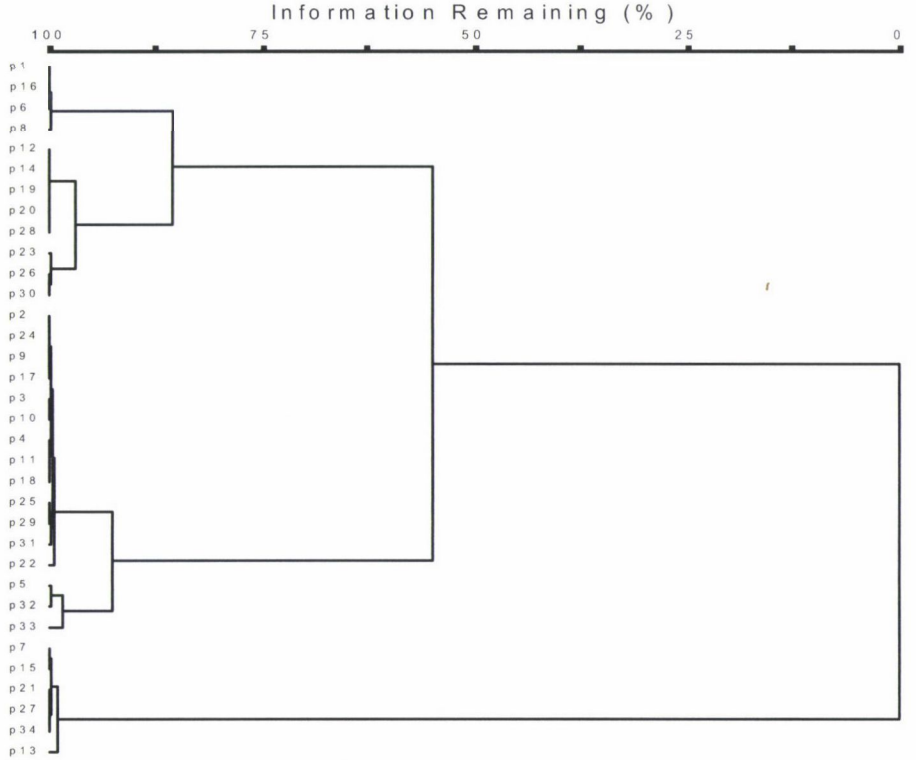

Fig. 1. Dendrogram of plots

In all the above plant communities the species *Halocnemum strobilaceum* is dominant, but the relative vegetation cover of the plant species or the concentration of dominance in the communities changes with changes in EC, pH, SAR and ESP and water table. Changes in the floristic composition and biodiversity of the communities also occur.

The analysis of vegetation and soil data using the CCA method shows the relationships between changes in soil factors and vegetation. The correlation of each environmental factor with the ordination axes is shown in Table 1. As can be observed from the table, the changes in the first axis are due to the water table and those in the second axis to other factors (SAR, ESP and pH). The third axis is under the influence of negative pH and positive EC. The correlation of the indicator species in the study region with the ordination axes is shown in Table 2.

As can be observed from Table 2, the species *Halocnemum strobilaceum* has the highest correlation with the first axis and then with the second axis, which is under the influence of a combination of SAR, ESP and pH. *Aeluropus littoralis* has a negative correlation with the second axis (i.e. a negative correlation with SAR, ESP and pH). This means that the plant favours soils with lower pH, SAR and ESP. The same is true for the species *Aeluropus lagopoides*, but with a lower coefficient. The species *Puccinella distans* has a high negative correlation with the first axis, indicating that the species has higher relative vegetation in the case of a high water table (about zero). Table 2 also shows a relatively high negative correlation with the first and second axes for *Salicornia europaea* (reverse of *Halocnemum strobilaceum*). The species *Salsola aurantia* has the highest negative correlation with the first axis only.

Table 1
CCA analysis (soil factors). Pearson and Kendall correlations with ordination axes (n=50)

Axis	1		2		3	
Environmental factor	r	r–sq	r	r–sq	r	r–sq
pH	–0.266	0.071	0.672	0.452	–0.660	0.436
EC	0.280	0.079	–0.207	0.043	0.331	0.110
ESP	0.093	0.009	0.789	0.623	–0.008	0.000
SAR	0.237	0.056	0.970	0.941	–0.189	0.036
WTL	0.856	0.733	0.414	0.172	–0.265	0.070

Table 2
CCA analysis (plant species). Pearson and Kendall correlations with ordination axes (n=50)

Axis	1		2		3	
Environmental factor	r	r–sq	r	r–sq	r	r–sq
Halocnemum strobilaceum	0.794	0.630	0.492	0.242	–0.092	0.009
Aeluropus littoralis	0.093	0.009	–0.671	0.450	0.245	0.06
Aeluropus lagopoides	–0.118	0.014	–0.357	0.128	–0.278	0.077
Puccinella distans	–0.800	0.640	0.328	0.108	–0.058	0.003
Salicornia europaea	–0.430	0.185	–0.443	0.197	–0.351	0.123
Salsola aurontiaca	–0.506	0.256	0.022	0.000	0.119	0.144

The relationships between the plant species and the soil factors were determined using Figs. 2, 3 and 4. Based on Table 2, the first and second axes are the most suitable for illustrating the relationship of *Halocnemum strobilaceum*, *Salsola aurantia*, *Salicornia europaea*, *Puccinella distans*, *Aeluropus lagopoides* and *Aeluropus littoralis* with soil factors.

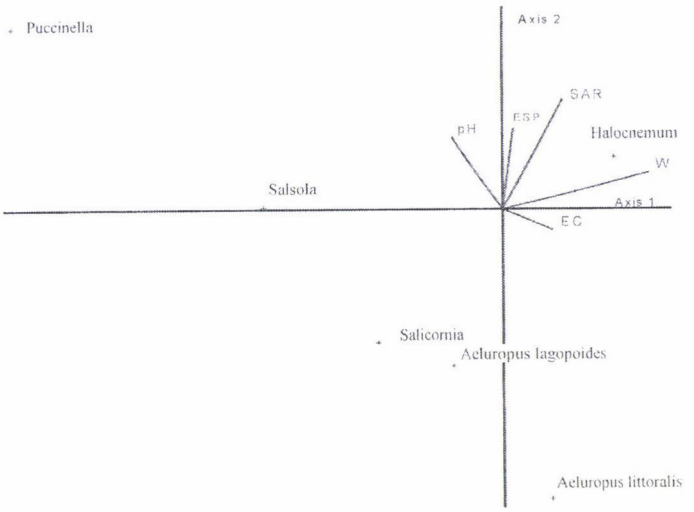

Fig. 2. Relationship of plant species with soil factors on x = 1 and y = 2 using the CCA method

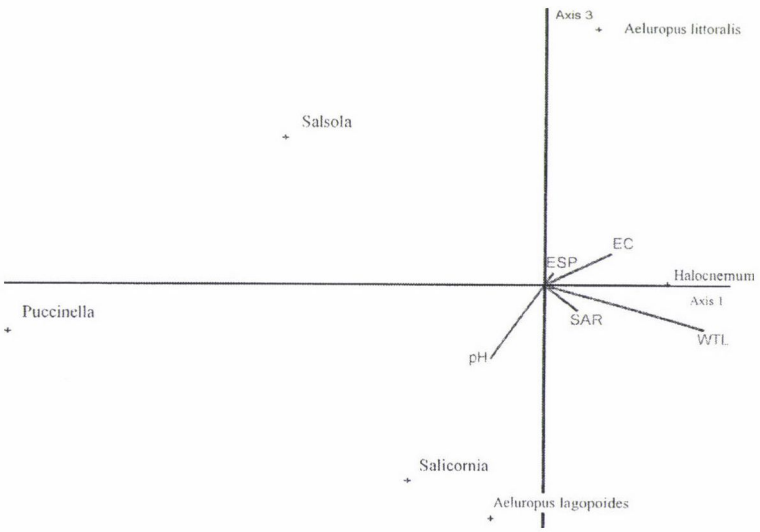

Fig.3. Relationship of plant species with soil factors on x = 1 and y = 2 using the CCA method

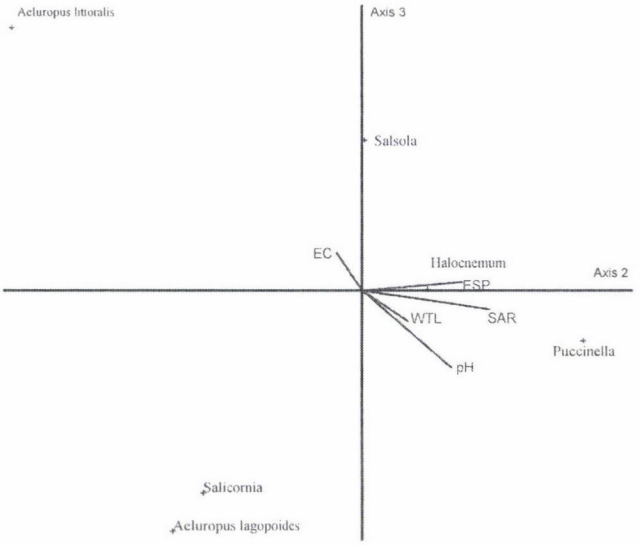

Fig.4. Relationship of plant species with soil factors on x = 2 and y = 3 using the CCA method

Discussion and recommendations

The species *Halocnemum strobilaceum* is the dominant vegetation of the region physionomically. This result confirms the findings of Ayoub and Malcolm (1993) and Malcolm and Choukr-Allah (1995) that *Halocnemum strobilaceum* is one of the most resistant plant species to salinity, sodicity and soil water-logging. However, in the present research the correlation of other plant species, such as *Aeluropus littoralis*, *Aeluropus lagopoides*, *Puccinella distans*, *Salsola aurontiaca* and *Salicornia europaea* with the species *Halocnemum strobilaceum* as a function of changes in ESP, SAR, pH, EC and water table has been detected in all the plant communities. These relationships are important for the creation of a sustainable physical environment and ecosystem. Changes in environmental factors not only create ecological niches for species with different ecological roles, but also have an influence on the formation of plant communities. Alakhverdiev (1988) stated that the distribution of *Aeluropus littoralis* along with *Halocnemum strobilaceum* is an indication of soil salinity and sodicity.

From the reports of FAO and the paper of Kovada (1980) it can be concluded that over 40% of the irrigated lands in Iran and Iraq are subject to secondary salinity (Pessarakli, 1993).

The salinity of soil and water is a threatening problem in agricultural production throughout the world. In Asia, for example, about 27 million ha is affected by soil or water salinity, so that little or no yield is obtained (Jafari, 1994). Advanced technology has not been successful in the reclamation of saline soils or in achieving sustainable production, and has made these fragile ecosystems more susceptible. The present research was an effort to understand the natural relationship between the components of these ecosystems. It has been observed that different plants are characteristically present in each set of ecological conditions. These plants have special functions, helping to make the ecosystems sustainable.

As these ecosystems are susceptible, any simplification or change in their use is recognised as the main factor in the spread of saline lands.

To protect and control saline soils and to make them sustainable, the following recommendations can be made:

- Considering the role and inherent functions of each ecosystem, saline soils should be considered from the environmental point of view (habitat, refuge, rangelands, etc.) and according to their roles in bio-geochemical cycles.
- Recommended methods for an increase in yield on saline soils should not consider only agronomic approaches. The use of saline soils as rangelands and special habitats for wild life should also be considered.
- As there is a direct relationship between the vegetation and factors such as SAR, ESP, pH, EC and water table, any change in the floristic composition may be the result of changes in these factors. In the control and management of these ecosystems, research and continuous inspection is recommended.
- In spite of the need for an increase in arable lands, saline lands should not be converted into agricultural lands, due to the need to protect biodiversity and sustain the functions of different ecosystems.

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EFFECT OF IRRIGATION REGIMES, MID-SEASON DRAINAGE AND TIME OF APPLICATION OF NITROGEN ON GROWTH AND YIELD OF HYBRID RICE

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Field experiments were conducted at the Central Farm of the Agricultural College and Research Institute, Madurai, India during the rabi (October–January) seasons of 1999–2000 and 2000–2001 in a split plot design with three replications. The soil of the experiments was sandy clay loam with a neutral reaction. The main plot consisted of six irrigation schedules with mid-season drainage, while four N splits were taken as sub- plots. The experimental results revealed that irrigation to a depth of 5 cm one day after the disappearance of ponded water and mid-season drainage, along with N applied in four splits, with 16.7% at 10 days after transplanting, 33.3% at active tillering, 33.3% at panicle initiation and 16.7% at the heading stage, produced significantly higher growth, yield attributes, grain and straw yields in hybrid rice. A combination of the above treatments led to maximum grain yields of 7533 and 8078 kg ha^{-1} (45.53 and 45.86% in excess of the control) in 1999–2000 and 2000–2001, respectively.

Key words: hybrid rice, irrigation, drainage, nitrogen splits, grain yield

Introduction

Rice is the staple food crop of densely populated Asian countries. India has a total production of 84.7 million tonnes of rice from 44.5 million hectares, with a productivity of 1903 kg ha^{-1} (Venkataramani, 2000). Any agricultural or water management techniques that can increase the productivity of rice ecosystems must be viewed as an important contribution to sustainable development. Drainage is very important for lowland rice, since it improves many physiological functions. Alternate wetting and drying along with mid-season drainage, especially at the maximum tillering stage, resulted in improved physiological functions in the rice crop and ultimately enhanced the grain yields. Growing hybrid rice is a complex process and the agronomic management, in particular, differs considerably from that of inbred rice varieties. New, front-line agronomic packages such as optimum plant population, seedling number $hill^{-1}$, optimum dose of N, split application of fertilizers and irrigation management, have a decisive effect on the yield potential of hybrid rice. Of these, irrigation and nitrogen management are considered to be the most important in influencing yields. Hence, the present study was carried out to discover the effect of irrigation schedule, drainage and the split application of N on medium duration hybrid rice.

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Materials and methods

The field experiments were conducted during the rabi (October–January) season of 1999–2000 and 2000–2001 at the Agricultural College and Research Institute, Madurai, on sandy clay loam soil with neutral pH (7.3). The experimental soil had low available N (156.4 kg ha^{-1}), medium available P (12.5 kg ha^{-1}) and medium available K (251.6 kg ha^{-1}), which were estimated by the alkaline permanganate method (Subbiah and Asija, 1956), Olsen's method (Olsen et al., 1954) and the normal N-ammonium acetate method (Jackson, 1973), respectively. The experiment was laid out in a split plot design with three replications. Hybrid rice CoRH 2 (125–130 days duration) was used for the study. The treatments in the main plots consisted of six irrigation schedules: I_1: Farmers' method of irrigation (continuous submergence to 5 cm depth); I_2: irrigation to 5 cm one day after the disappearance of ponded water; I_3: irrigation to 5 cm three days after the disappearance of ponded water; I_4: farmers' method of irrigation with mid-season drainage at the maximum tillering stage (40–45 DAS); I_5: irrigation to 5 cm one day after the disappearance of ponded water with mid-season drainage at the maximum tillering stage (40–45 DAS); I_6: irrigation to 5 cm three days after the disappearance of ponded water with mid-season drainage at the maximum tillering stage (40–45 DAS).

The subplots consisted of four N application splits: N_1: in three equal splits, with 33.3% each at 10 days after transplanting, active tillering and panicle initiation; N_2: four splits with 16.7% at 10 days after transplanting + 33.3% at active tillering + 33.3% at panicle initiation + 16.7% at heading; N_3: four splits with 25% each at 10 days after transplanting, active tillering, panicle initiation and heading; N_4: five equal splits with 20% each at 10 days after transplanting, active tillering, maximum tillering, panicle initiation and heading.

The recommended dose of fertilizers (150: 50: 50 N, P_2O_5 and K_2O kg ha^{-1}) were applied as urea, single superphosphate and muriate of potash. The planting was done with a spacing of 20 × 10 cm with a single seedling $hill^{-1}$. The other cultivation practices were those normally recommended for the crop (Crop Production Guide, 1999). The fertilizer nitrogen was applied in the form of prilled urea (46% N) in three, four and five splits as per the treatment schedule. The entire phosphorus fertilizer was applied as basal in the form of single superphosphate (16% P_2O_5). The potassium fertilizer was applied in the form of muriate of potash (60% K_2O) in three splits at tillering, panicle initiation and heading. Due to a consideration of the previous results, basal N application was not included in the treatments.

For the purpose of observations, samples consisting of five plants were selected at random and tagged. Growth parameters [plant height, number of tillers $hill^{-1}$, leaf area index (LAI), dry matter production (DMP)] and yield attributes [number of panicles $hill^{-1}$, panicle length (cm), panicle weight (g), number of grains $panicle^{-1}$, thousand grain weight] were recorded. Plant height was measured from the surface of the soil to the tip of the topmost leaf at harvest. The number of tillers $hill^{-1}$ was recorded at the maximum tillering stage. LAI was estimated at harvest using the formula suggested by Yoshida et al. (1976).

Plant samples were taken at harvest, dried in a hot air oven at 80°C ± 5°C for 48 hours, and the oven dry weight was computed to t ha^{-1}. The number of panicles $hill^{-1}$ was counted at harvest. The panicles collected from the sampling area were used for recording the weight. The samples were sun-dried to constant weight, expressed in g $panicle^{-1}$. Thousand-grain weight was recorded in grams by weighing the grain obtained from the samples at 14% moisture (Yoshida et al., 1976). The matured crop was harvested from the net plot area and the grains were hand threshed, winnowed and sun-dried. The dried grains from each plot were weighed and computed to t ha^{-1}. The straw yield was calculated after threshing the grains by sun-drying the straw from each plot, weighing it and converting it to t ha^{-1}. The data were subjected to statistical analysis (Panse and Sukhatame, 1978).

Results and discussion

Growth parameters

The results of the experiment revealed that both irrigation and N management practices (Table 1) significantly influenced the growth parameters, namely plant height, number of tillers hill^{-1}, leaf area index (LAI) and dry matter production (DMP). In both years the plant height at harvest was greater (102.3 and 109.4 cm) when irrigation was carried out to a depth of 5 cm one day after the disappearance of ponded water in combination with mid-season drainage than in the other irrigation treatments. The increased plant height observed for this treatment was due to favourable root growth and the higher mobility of N in the soil solution, leading to the greater uptake of N by the rice plants. The number of panicles hill^{-1} (15.75 and 16.85), LAI (5.68 and 6.07) and DMP (15350 and 16424 kg ha^{-1}) were also maximum in this treatment (I_5). The increase in LAI may have been caused by the increased plant height and number of tillers hill^{-1}. Better aeration due to mid-season drainage may have resulted in favourable root growth and absorption of nutrients. The increased plant height, number of tillers hill^{-1} and LAI registered in treatment I_5 contributed to the increased DMP. This is in agreement with the findings of Raju et al. (1993) and Chandrasekaran (1996). Similarly, Vaithiyalingam (1984) also reported increased growth characters due to drainage.

Table 1
Effect of irrigation schedule and time of N application on growth characters

Treatment	Plant height (cm)		LAI		DMP (kg ha^{-1})	
	1999–2000	2000–2001	1999–2000	2000–2001	1999–2000	2000–2001
Main plot						
Irrigation schedule						
I_1	94.85	101.48	4.53	4.84	14980	16028
I_2	96.91	103.69	4.77	5.10	15000	16050
I_3	93.25	99.77	4.34	4.64	14979	16028
I_4	99.75	106.73	5.21	5.57	15168	16230
I_5	102.25	109.40	5.68	6.07	15350	16424
I_6	90.83	97.18	4.19	4.48	14125	15114
SEd	0.318	0.340	0.02	0.02	249	266
CD (P = 0.05)	0.709	0.758	0.03	0.04	555	594
Sub-plot						
N split						
N_1	95.18	101.18	4.68	5.00	14587	15769
N_2	97.22	104.02	4.87	5.20	15256	16215
N_3	96.45	103.60	4.85	5.19	15086	16119
N_4	96.37	103.37	4.74	5.07	14807	15812
SEd	0.44	0.45	0.03	0.03	67	72
CD (P = 0.05)	0.86	0.92	0.05	0.05	136	146

The application of N in four splits either as 16.7% at 10 days after transplanting, 33.3% at active tillering, 33.3% at panicle initiation and 16.7% at heading, or 25% each at 10 days after transplanting, active tillering, panicle initiation and heading proved better with respect to plant height, number of tillers $hill^{-1}$ and LAI. The increase in growth characters was mainly due to the better absorption and utilization of N, as observed from the higher uptake in these treatments. The increase in these growth characters consequently resulted in higher DMP. The beneficial effect of four splits made at 10 days after transplanting, active tillering, panicle initiation and heading was also reported by Palanimurugesan (1997).

Irrigation to a depth of 5 cm one day after the disappearance of ponded water with mid-season drainage, in combination with N applied in four splits (16.7% at 10 days after transplanting, 33.3% at active tillering, 33.3% at panicle initiation and 16.7% at heading) resulted in higher growth parameters as compared to all other combinations. This may be due to the favourable physico-chemical properties created by this irrigation schedule, while the N splits may have influenced the N uptake and in turn increased the growth parameters of rice.

Yield attributes

The yield attributes of rice were significantly influenced by both irrigation and N management practices (Table 2). Submergence to a depth of 5 cm one day after the disappearance of ponded water, combined with mid-season drainage, resulted in the maximum number of panicles $hill^{-1}$ (13.00 and 14.05), panicle length (29.00 and 31.03 cm), panicle weight (4.02 and 4.30 g), number of grains $panicle^{-1}$ (114 and 122) and test weight (22.49 and 24.19 g) in the two years. This may have been due to the better aeration of the root system, associated with the higher mobility and absorption of inorganic N in the soil solution, which increased the uptake of nutrients and contributed to favourable growth attributes, which in turn resulted in higher yield attributes. This is in agreement with the findings of Palchamy et al. (1989).

The fractional application of N extending up to heading was beneficial. The lesser quantity (16.7%) of N at 10 days after transplanting and at heading and the increased dose (33.3%) of N at active tillering and panicle initiation resulted in higher values of number of panicles $hill^{-1}$ (11.08 and 12.06), panicle length (24.09 and 25.70 cm), panicle weight (3.33 and 3.56 g), number of grains $panicle^{-1}$ (101 and 108) and test weight (22.42 and 24.10 g) in both years of experimentation. The synchronization of N uptake with crop demand in this treatment may have been conducive to the translocation of carbohydrates to the sink, resulting in favourable growth, which in turn was reflected in the yield attributes. Similar findings were reported by Palanimurugesan (1997).

The combined application of irrigation to a depth of 5 cm one day after the disappearance of ponded water with mid-season drainage and N applied in four splits (16.7% at 10 days after transplanting, 33.3% at active tillering, 33.3% at panicle initiation and 16.7% at heading) gave maximum values of yield attributes in rice in both seasons. This could have been due to the improvement in nutrient uptake from the better soil environment, which had an influence on growth characters and ultimately increased the yield attributes of rice.

Grain and straw yield

The grain and straw yield of rice were significantly influenced by the irrigation and N treatments (Table 3). The maximum grain yield (7064 and 7556 kg ha^{-1}) and highest straw yield (9745 and 10428 kg ha^{-1}) were obtained due to the judicious combination of irrigation to a depth of 5 cm one day after the disappearance of ponded water with mid-season drainage. Mid-season drainage may have arrested the growth of late tillers and ultimately resulted in the efficient conversion of total tillers to number of panicles. The favourable growth with higher nutrient uptake, along with the increased yield attributes, resulted in higher grain yield. Similar findings were reported by Ramamoorthy et al. (1993). Ghosh and Das (1999) also reported the advantage of mid-season drainage at maximum tillering on the grain yield of rice.

The grain and straw yields of hybrid rice were greatly influenced by the split application of N. The application of N in four splits (16.7% at 10 days after transplanting, 33.3% at active tillering, 33.3% at panicle initiation and 16.7% at heading) or the balanced application of 25% each at 10 days after transplanting, active tillering, panicle initiation and heading produced higher rice grain and straw yields and were comparable with each other. The percentage increase in grain yield in these treatments was 15.8 and 14.7% (1999–2000 and 2000–2001) and 15.6 and 15.0% (1999–2000 and 2000–2001), respectively, compared with three splits. The increased yields achieved with four splits might be due to the increased uptake of nutrients, coupled with increased growth and yield attributes. Similar findings were reported by Palanimurugesan (1997) and Sivakami (2000).

From the above results, it can be concluded that the combination of irrigation to a depth of 5 cm one day after the disappearance of ponded water, mid-season drainage and N in four splits (16.7% at 10 days after transplanting, 33.3% at active tillering, 33.3% at panicle initiation and 16.7% at heading) resulted in higher grain and straw yields in rice. The better physico-chemical environment prevailing under these treatment conditions was conducive for the production of favourable growth and yield attributes, which in turn was reflected in higher grain yields.

Table 2
Effect of irrigation schedule and time of application of N on yield attributes

Treatment	No. of panicles hill^{-1}		Panicle length (g)		Panicle weight (g)		No. of grains per panicle		Test weight (g)		Uptake of N (kg/ha)	
Main plot Irrigation schedule	1999–2000	2000–2001	1999–2000	2000–2001	1999–2000	2000–2001	1999–2000	2000–2001	1999–2000	2000–2001	1999–2000	2000–2001
I_1	9.75	10.57	21.99	23.52	3.02	3.23	94	101	21.60	23.25	98.37	105.25
I_2	11.00	11.91	23.75	25.41	3.25	3.47	100	107	21.97	23.64	101.67	108.87
I_3	9.00	9.77	20.75	22.20	2.83	3.02	89	95	21.13	22.74	96.72	103.49
I_4	12.09	13.07	26.00	27.82	3.61	3.86	108	115	22.14	23.82	104.32	111.62
I_5	13.00	14.05	29.00	31.03	4.02	4.30	114	122	22.49	24.19	107.97	115.52
I_6	7.25	7.89	19.00	20.33	2.63	2.81	84	90	20.59	22.17	94.77	101.48
SEd	0.49	0.52	0.52	0.55	0.01	0.01	2.31	2.47	0.13	0.13	0.208	0.222
CD (P=0.05)	1.10	1.18	1.14	1.21	0.02	0.02	5.15	5.51	0.28	0.29	0.464	0.496
Sub-plot, N split												
N_1	9.23	10.01	22.50	24.07	3.11	3.32	96	102	20.76	22.35	99.75	106.73
N_2	11.08	120.6	24.09	25.70	3.33	3.56	101	108	22.42	24.10	101.60	108.71
N_3	10.92	11.85	23.89	25.63	3.27	3.49	99	107	21.31	24.03	100.88	107.94
N_4	10.17	11.02	23.17	24.71	3.19	3.41	97	106	21.31	22.71	100.33	107.35
SEd	0.14	0.15	0.09	0.09	0.03	0.03	0.04	0.04	0.24	0.25	0.348	0.372
CD (P=0.05)	0.29	0.31	0.20	0.21	0.07	0.07	0.08	0.09	0.48	0.51	0.767	0.820

Table 3
Effect of irrigation schedule and time of N application on grain yield (kg ha^{-1})

Treatment	Irrigation schedule													
	1999–2000							2000–2001						
Split application of N	I_1	I_2	I_3	I_4	I_5	I_6	Mean	I_1	I_2	I_3	I_4	I_5	I_6	Mean
N_1	5176	5851	5329	6321	6382	5160	5703	5538	6260	5702	6763	6829	5521	6102
N_2	6520	6861	6847	7112	7533	6100	6775	6993	7358	6755	7628	8078	6725	7235
N_3	6478	6664	6294	7199	7440	6301	6689	6914	7113	6717	7685	7943	6545	7183
N_4	6003	6096	5873	6423	6900	5643	6156	6424	6522	6284	6873	7383	6038	6587
Mean	6044	6368	5961	6773	7064	5776		6467	6814	6378	7236	7558	6207	

Treatments	1999–2000		2000–2001	
I	160	356	171	380
N	49	99	48	106
I at N	190	413	204	442
N at I	120	243	128	260

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EVALUATION OF GENETIC STOCKS DERIVED FROM *HORDEUM VULGARE* L. × *H. BULBOSUM* L. CROSSES

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The aim of the programme started at ARDI-Fundulea in 1999 is to improve the pest and disease resistance of cultivated barley (*H. vulgare* L.) by introgressing valuable genes from the wild species *H. bulbosum* L.

The paper presents results on the development and cytogenetical characterization of primary genetic stocks represented by diploid, triploid and tetraploid interspecific hybrids and first backcrossed generation descendants.

Several sterile diploid hybrids were found during the phenotypic screening and cytological analysis of haploid progeny from *H. vulgare* 2x × *H. bulbosum* 2x crosses. These hybrids were treated with colchicine and fertile tetraploid hybrids were obtained.

Significant improvements in the seed setting and *in vitro* triploid hybrid regeneration were obtained using doubled haploid lines (DHLs), previously selected for high interspecific crossability, in crosses with a tetraploid cytotype of *H. bulbosum*. Meiosis analysis of triploid hybrids provided compelling evidence that relatively high intergenomic allosyndetic pairing had occurred in some of the triploids with increased potential for crossing over and genetic recombination.

High mean values for hybrid stability, multivalent associations in MI, higher chiasma frequency per PMC and partial pollen fertility were considered by far the most important criteria in the cytogenetic selection of triploid hybrids. Selected triploids were backcrossed to barley DHLs. Among the *in vitro* regenerated backcrossed progeny several putative substitution lines (SLs) were identified by preliminary cytological screening. The complete phenotypic and cytogenetic characterization and disease resistance tests of tetraploid hybrids and putative SLs or RLs are now in progress.

Key words: barley DHLs, *Hordeum bulbosum*, triploids, cytogenetical selection, BC_1

Introduction

Besides haploid induction, an important aim of interspecific hybridization between *H. vulgare* L. and the wild species *H. bulbosum* L., has been the introgression of desirable genes into the barley genome. The biotechnological *bulbosum* system for haploid production is based on the complete elimination of *bulbosum* chromosomes in *H. vulgare* (2x) × *H. bulbosum* (2x) crosses, followed by doubling the chromosome number and the release of doubled haploid lines (DHLs) (Lange, 1971).

Gene introgression is based on the retention of some or all of the *H. bulbosum* chromosomes in crosses between diploid and tetraploid cytotypes of both species with high potential for intergenomic pairing (Pickering et al., 2000).

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H. bulbosum L. is a wild, perennial, bulbous barley grass of winter habit, native to the Fertile Crescent and the Mediterranean regions (Bothmer et al., 1995). It is an obligatory outcrossing and self-incompatible species, with diploid and tetraploid cytotypes. *H. bulbosum* is considered to be the most closely related species to cultivated barley and is the single component of the second gene pool. *H. bulbosum* possesses useful agronomic traits such as: allogamy with the shedding of a large amount of pollen, hairy leaf sheath and limbs, resistance to abiotic stress (winter hardiness and drought tolerance) and to the major fungal and viral pathogens. It also shows resistance to Russian wheat aphid (Zeller, 1998; Walther et al., 1999).

Alien gene transfer in barley has been difficult to achieve because of the rarity of closely related species and of its eudiploid constitution, which prevents extensive chromosome engineering. Successful gene transfer from wild species into barley has until recently been limited to crosses between *H. vulgare* and *H. vulgare* ssp. *spontaneum* (Lehmann, 1991).

In hybridization with *H. bulbosum* gene introgression has been impeded by several pre- and post-pollination barriers. These are crossing incompatibility, chromosome instability, diminution or absence of chromosome pairing, low frequency of crossing-over, hybrid infertility and pollen tube competition. In the last decade real progress in overcoming these barriers has been made (Mihailescu and Giura, 2000; Pickering, 1991). It seems that the incompatibility in crosses between *H. vulgare* and *H. bulbosum* is controlled by a single dominant gene located in the barley genome. This has in part been resolved by selecting parental genotypes and by using *in vitro* embryo culture. Chromosome instability in the developing embryos and in various *H. vulgare* × *H. bulbosum* hybrids is the result of *H. bulbosum* chromosome elimination controlled by genes located on barley chromosomes 2H and 3H (Ho and Kasha, 1975).

H. bulbosum chromosomes can be retained by manipulating the genome ratio and by allowing embryos to develop below 18°C. Genome ratios of 1V:1B, 2V:1B and 2V:2B favour chromosome elimination, whereas 1V:2B results in chromosome retention and hybrid production (where V and B are *H. vulgare* and *H. bulbosum* genomes, respectively). Intergenomic homoeologous pairing varies considerably from complete absence in VVB hybrids to partial allosyndesis in VB and VBB hybrids (Mihailescu and Giura, 1999a; Pickering, 1992). The fertility of these interspecific hybrids can be restored by doubling the chromosome number of the diploids to obtain tetraploids (VVBB) or by developing partially fertile triploids (VBB) to be backcrossed to barley. The rarity of crossing-over between paired homoeologous chromosomes of the two species remains the principal barrier obtaining genetic recombination.

However, successful gene transfers from wild species into cultivated barley have already been reported. A plant with resistance to powdery mildew was obtained by Xu and Kasha (1992). Pickering et al. (2000) established the fact that *H. bulbosum* DNA containing the powdery mildew resistance gene

(Mlhb) had been introgressed into chromosome 2H(2). Pickering's research team also developed new recombinant lines (RLs) with improved resistance to mildew, leaf rust and scald and with useful morphological traits. The aim of the programme started at ARDI-Fundulea in 1999 is to improve the pest and disease resistance of cultivated barley by introgressing genes from *H. bulbosum*. The paper presents results on the development, cytogenetical characterization and utilization of primary genetic stocks: diploid, triploid and tetraploid hybrids, BC_1 generation and putative substitution lines (SLs) derived from *H. vulgare* × *H. bulbosum* crosses.

Materials and methods

The most efficient crosses between diploid and tetraploid cytotypes of *H. vulgare* L. and *H. bulbosum* L. carried out at ARDI-Fundulea were the following: *H. vulgare* 2x × *H. bulbosum* 2x and *H. vulgare* 2x × *H. bulbosum* 4x. In some of these crosses, barley doubled haploid lines (DHLs) were used as maternal parent. The use of selected barley DHL genotypes, which are completely homozygous, was an attempt to counteract the genetic control of the *vulgare* parent on crossability (one dominant gene, *Inc* located on 5HS) and on homoeologous chromosome pairing in crosses with *H. bulbosum*.

Thus, triploid hybrids were produced by crossing 8 barley DHLs (DHL 32-16; -17; -19; -22; -24; -27; -28 and DHL 32-29) derived from the two-row winter variety Andra (released at ARDI-Fundulea) with a natural tetraploid *H. bulbosum* cytotype. The material used for the first backcross comprised 6 barley genotypes (4 DHLs: DHL-44, DHL-45, DHL-72, DHL-104, and the two-row spring varieties Marina and Ottis) and 7 partially fertile triploid hybrids (THs) including the cytogenetically selected ones.

Interspecific hybridization for the development of triploid hybrids and the BC_1 generation was made in the field from the second half of May to early June. Combined *in vivo* treatments with plant growth regulators were applied at 24 h and 48 h after pollination. Hybrid and BC_1 plants were regenerated *in vitro* by embryo culture. Perennial triploid hybrids (VBB) were transferred to the field after a preliminary phenotypic screening based on several morphological marker traits. Plantlets were scored for sheath leaf and stem pubescence, leaf width, habit and vegetative type. Awn/spike length ratio has been recognised as a very good discriminating trait for the preliminary screening of *H. vulgare* × *H. bulbosum* progenies.

The crossability and regeneration of *in vitro* hybrids and BC_1 plants were estimated by many parameters: S/S (seed set), SD/F (dissected seeds to 100 pollinated florets), E/F (embryos to 100 pollinated florets), E/SD (embryos to 100 dissected seeds), EG/EC (germinated embryos to 100 cultivated embryos), TH, BC_1/F (triploid or BC_1 plants to 100 pollinated florets), TH, BC_1/EC (triploid or BC_1 plants to 100 cultivated embryos) and TH, BC_1/sp (triploid or BC_1 plants to one spike).

Mitotic analysis was made by the Feulgen method in the roots of hybrids and backcrossed plantlets. By counting the somatic chromosome number and the satellited chromosomes, the hybrid type and genomic structure were established. Diploid (VB, 2n=2x=14) and triploid (VBB, 2n=3x=21) hybrids showed only two SAT-chromosomes: $5H^V(7)$ and $6H^V(6)$ due to the amphyplasty exercised by the *H. vulgare* genome. For meiotic analysis the spikes were fixed in Carnoy's solution and the anthers were squashed in aceto-carmine. Hybrid stability (percentage of PMCs with chromosome number of 2n=2x=14 or 2n=3x=21), frequency and configuration of chromosome associations at MI, mean chiasma frequency per PMC, pairing chromosome capacity, percentage of PMCs with autosyndetic pairing and pollen fertility in one diploid (215 PMCs) and 7 triploid hybrids (from 60 to 160 PMCs) were considered. Pollen fertility was determined by the aceto-carmine test.

Results and discussion

The 29 putative diploid hybrids occurring among the haploid progenies (*H. vulgare* 2x × *H. bulbosum* 2x) were separated by phenotypic screening. Out of these, 75.9% were haploid (V, 2n=x=7), 3.4% diploid (VB, 2n=2x=14) and 3.5% mixoploid, while 17.2% had no mitotic division.

The diploid hybrid had high cytological stability (99.5% of PMCs with 2n=2x=14) with low intergenomic pairing (at MI: 4.2 I + 4.6 II + 0.1 III + 0.02 IV) and a low chiasma frequency per PMC of 6.4. These data are comparable with previously published results (Lange, 1971; Pickering, 1988; Xu and Snape, 1988). The diploid hybrids were treated with colchicine to double the chromosome number.

Significant improvements in crossability and *in vitro* hybrid regeneration were obtained by using selected winter two-row doubled haploid lines (DHLs) in crosses with a natural tetraploid cytotype of *H. bulbosum*. The mean values for the crossability and *in vitro* regeneration parameters were constantly higher in crosses with DHLs compared to breeding lines and varieties (Table 1).

The values for S/S varied from 57.2 to 86.2 (mean value 71.4) with DHLs compared to 38.4–45.4 with lines and varieties, while the TH/EC values ranged from 65.6 to 87.7 (mean value 71.7) compared to 17.6–23.6, respectively. In crosses with lines or varieties as maternal parents only 41 hybrids were regenerated compared to 744 hybrids with DHLs. The results also showed significant differences compared to previously reported data. Pickering (1988) obtained mean values of 33.1 for S/S and 4.3 for TH/EC, with 45 regenerated hybrids. Xu and Kasha (1992) reported values of 21.9 for S/S and 0.6 for TH/EC, with 12 regenerated hybrids.

Hybrid plantlets were phenotypically screened based mainly on *bulbosum* "marker" traits (hairy leaf sheaths, limbs and stem, habit type, etc.). In the adult stage, the determination of the awn/spike length ratio became a useful way to discriminate between parental and hybrid genotypes (Table 2). The mean value of the awn/spike length ratio was slightly above 2.0 (1.87–2.26) for *H. vulgare* and around 1.0 (1.06–1.10) for *H. bulbosum*. The diploid hybrid (VB) had a ratio closer to the *H. vulgare* value (2.03), while that of the triploid hybrids (VBB) was somewhat higher than that of the *H. bulbosum* parent (1.20–1.32). Ratio values of 2.10 for *H. vulgare* and 2.3–2.7 for diploid hybrids were also reported by Pickering (1992).

In a sample of 59 triploid descendents (*H. vulgare* 2x × *H. bulbosum* 4x) mitotic analysis showed that 96.5% were triploids (VBB, 2n=3x=21) and 3.5% possible haploids (unclear mitotic divisions).

In addition to the selective elimination of bulbosum chromosomes, the suppression of homoeologous allosyndetic pairing and of the nucleolar constriction of *H. bulbosum* chromosomes exercised by the *vulgare* genome in interspecific crosses made it necessary to select diploid and triploid hybrids cytogenetically for gene transfer.

Table 1
Crossability and *in vitro* hybrid regeneration in *H. vulgare* L. 2x × *H. bulbosum* L. 4x crosses

Genotype	S/S	E/F	EG/EC	TH/F	TH/EC
7 lines/varieties	38.4–45.4	7.7–18.9	35.3–46.1	3.5–3.7	17.6–23.6
DH32–16	86.2	54.5	90.9	38.4	70.5
–17	83.2	63.6	98.1	41.6	66.7
–19	84.3	68.6	98.0	45.0	65.6
–22	74.3	59.2	99.0	59.2	87.7
–24	71.1	52.5	96.5	34.5	65.8
–27	58.6	34.8	98.4	25.4	73.0
–28	66.4	62.3	100.0	50.7	81.3
–30	57.6	38.7	96.1	28.2	74.4
Mean	71.4	52.4	96.9	37.6	71.7

Table 2
Awn/spike length ratio of parents and hybrids

Lines	Spike length x ± sx	Awn length x ± sx	Ratio
H. vulgare (VV)			
Marina*	9.83±1.02	22.20±1.42	2.26
Ottis*	10.13±0.71	22.19±1.09	2.19
DH 32–30*	11.04±0.65	20.66±0.77	1.87
DH 98–19**	10.77±0.94	23.16±1.26	2.15
H. bulbosum (BB)			
H. bulbosum 2x	10.13±1.02	10.70±1.17	1.06
H. bulbosum 4x	13.64±1.25	14.97±1.19	1.10
Hybrids, 2n=14 (VB) and 2n=21 (VBB)			
VB hybrid–DH	7.83±0.60	15.92±0.90	2.03
VBB hybrid –TH1	12.17±1.68	14.97±2.09	1.22
– TH2	13.22±1.63	15.76±1.98	1.20
– TH3	9.34±1.43	12.35±1.78	1.32

*: two-row; **: six-row barley

A particularly distinctive meiotic feature of triploid hybrids was the constant presence (from prophase to pollen cells) of chromatinic/chromatidic inclusions/fragments with an increased incidence of hypoaneutriploids. These events were determined by the elimination of the *bulbosum* chromosomes and the auto-allo-genomic structure (VBB) of the triploids.

Out of the seven triploid hybrids analysed, four had 2n=3x=21, two 2n=3x=20 and one 2n=3x=19. Cytological stability values varied from 27.9% (TH-51) to 99% (TH-13). MI chromosome associations per PMC showed large variation among the hybrids: 4.4–5.7 I; 0.7–1.5 rod II; 3.6–4.8 ring II; 0.7–2.2 III; 0.02–0.2 IV and 0–0.03 V (Fig. 1). The mean chiasma frequency per PMC ranged from 10.82 (TH-87) to 15.5 (TH-28) and the intergenomic chromosome pairing capacity from 70.5% (TH-13) to 80.6% (TH-28) (Table 3). Autosyndetic PMCs varied from 7.14% (TH-14) to 45.22% (TH-13). All these data provided compelling evidence that relatively high intergenomic allosyndetic chromosome

pairing had occurred in some of the triploids, with increased potential for crossing over and genetic interspecific recombination. Also, in a few triploids some specific abnormality characterized the chromosome rearrangements. The pollen fertility of the triploid hybrids varied from 11.7% to 34.0%. Autosyndetic PMCs varied from 7.14% (HT-14) to 45.22% (HT-13).

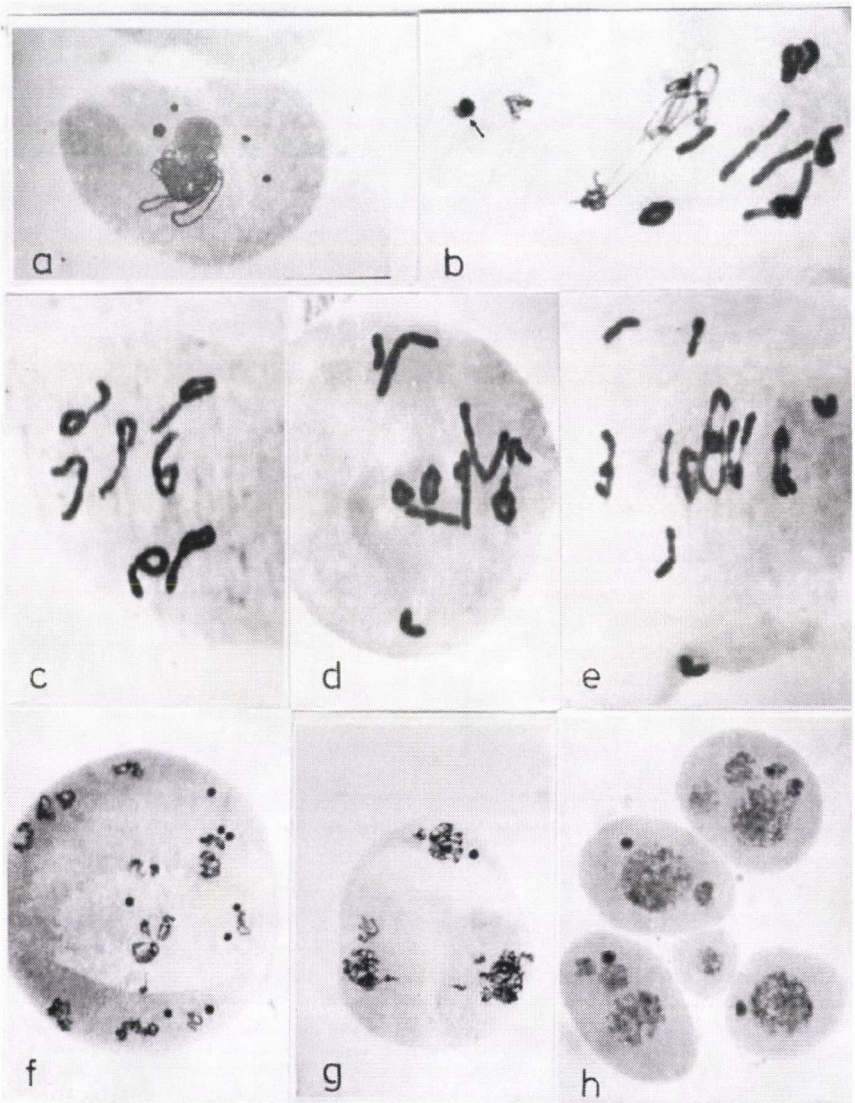

Fig. 1. Meiosis in triploid hibrids, VBB (2n=3x=21). a) Prophase with inclusions and nucleole. b) MI with inclusion (arrow) and uncondensed chromosomes at a stage equivalent to leptotene-pachytene. c) MI: 7 III. d) MI: 2 I +2 II rod +3 II ring + 3 III. e) MI: 5 I +2 II rod + 1 II ring + 2 III + 1 IV. f) "Uniad" with multipolar disjunctions and micronuclei. g) Triad with micronuclei. h) Micrograins of various sizes

High mean values of hybrid stability, multivalent chromosome association at MI, higher chiasma frequency per PMC, low percentage of autosyndetic PMC and partial pollen fertility were considered by far the most important criteria for the cytogenetic selection of triploid hybrids. Triploid hybrids TH-13, TH-14, TH-16, TH-25 and TH-28 were selected for backcrosses to DHLs of cultivated barley. Similar meiotic MI configurations were found by many other researchers. Lange (1971) reported MI: 5.8–6.8 I + 5.5–6.8 II + 0.13–1.3 III + 0.03–0.7 IV; Xu and Kasha (1992) found MI: 5.5 I + 5.3 II + 1.5 III + 0.01 IV and Gilpin et al. (1997) MI: 4.4 I + 5.3 II + 1.6 III + 0.03 IV for triploids. The pollen fertility of the triploid hybrids varied from 11.7% to 34.0%.

In backcrosses of partially fertile triploids to barley DHLs, high significant mean values for crossability and *in vitro* plant regeneration were obtained (Table 4). The mean values for the SD/F, E/F and EG/EC parameters were 81.5, 34.0 and 84.0, respectively. The 475 regenerated BC_1 plants represented 62.2% of the *in vitro* germinated embryos (BC_1/EG) and 18.3% of the pollinated florets (BC_1/F). The BC_1 seeds had a predominantly watery endosperm, but several had a partially solid endosperm. Out of 157 cytologically investigated BC_1 plants 28.7% (45 plants) had 2n=14, 68.1% (107 plants) were haploids and 3.2% (5 plants) had an unknown chromosome number. Four diploid plants were regenerated from embryos of seeds with a partially solid endosperm and 6 other plants exhibited hairy leaves and stems. These results were in very good agreement with data already published (Pickering, 1992). The complete phenotypic characterization, cytogenetic and molecular analysis and disease resistance tests of tetraploid hybrids and putative substitution lines (SLs) or recombinant lines (RLs) are now in progress.

Table 3
Metaphase I analysis of triploid hybrids

Hybrids	I	II			III	IV	V	Chi/PMC
		Total	Rod	Ring				
TH-13	6.2 (2–9)	5.6	0.8 (0–4)	4.8 (0–7)	1.2 (0–5)	0.03 (0–1)	–	13.13
TH-14	4.4 (0–8)	5.5	1.0 (0–4)	4.5 (2–8)	1.8 (0–5)	0.07 (0–1)	–	14.16
TH-16	5.3 (2–8)	5.7	1.2 (0–5)	4.5 (0–7)	1.3 (0–5)	0.06 (0–1)	–	13.23
TH-25	4.4 (0–7)	5.3	0.7 (0–3)	4.8 (1–8)	1.7 (0–6)	0.11 (0–1)	0.03 (0–1)	14.45
TH-28	3.9 (0–8)	4.4	0.8 (0–5)	3.6 (0–8)	2.2 (0–6)	0.2 (0–3)	0.02 (0–1)	15.48
TH-51	4.2 (0–10)	5.5	1.5 (0–4)	4.1 (0–7)	0.7 (0–3)	0.03 (0–1)	–	11.26
TH-87	5.2 (0–10)	5.1	1.5 (0–5)	3.6 (1–6)	1.0 (0–2)	–	–	10.82

Cytological stability						
TH–13	TH–14	TH–16	TH–25	TH–28	TH–51	TH–87
2n = 21	2n = 21	2n = 21	2n = 21	2n = 20	2n = 19	2n = 20
99.1%	94.3%	84.5%	95.4%	88.6%	27.9%	31.6%

Table 4
Crossability and *in vitro* BC_1 plant regeneration

Lines	SD/F	E/F	EG/EC	BC^1/EG	BC^1/F
Marina	69.0	9.8	91.7	36.4	3.3
Ottis	76.7	42.2	95.4	56.6	22.8
DH–44	91.6	49.3	80.1	66.0	26.0
DH–45	82.2	65.6	97.1	77.0	49.0
DH–72	86.2	73.2	98.0	62.6	44.9
DH–104	80.0	20.8	65.5	53.4	7.18
Mean	81.5	34.0	84.0	62.2	18.3

Pickering (1992): SD/F = 0.2–29.2; Xu and Snape (1987) SD/F = 9.7–45.8; E/F = 5.4–27.2

Conclusions

A significant increase in crossability and in the stability of cytological triploid hybrids were achieved using selected doubled haploid lines (DHLs) of two-row winter barley in crosses with a natural tetraploid cytotype of *H. bulbosum.*

Meiotic analysis provided compelling evidence that in some of the triploid hybrids high intergenomic allosyndetic chromosome pairing had occurred with increased potential for crossing-over and genetic interspecific recombination between *H. vulgare* and *H. bulbosum.*

Cytogenetically selected, partially fertile triploid hybrids, used as pollen sources in backcrosses to barley doubled haploid lines (DHLs), showed high crossing compatibility and *in vitro* regeneration of BC_1 plants.

Among the *in vitro* regenerated backcrossed progeny several putative substitution lines have been identified by means of phenotypical and cytological screening.

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COMBINING ABILITY FOR PHYSIOLOGICAL TRAITS IN SPRING WHEAT OVER ENVIRONMENTS

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Combining ability analysis was carried out in the F_1 and F_2 generations of a 10 × 10 parents half diallel for peduncle length and flag leaf area in spring wheat under three environments. The mean squares for both general combining ability (GCA) and specific combining ability (SCA) were significant for peduncle length in both the generations, indicating the involvement of both additive and non-additive gene actions in the inheritance. However, the high values of GCA variance showed the greater importance of additive gene action in the inheritance of this trait. Flag leaf area was observed to be controlled by non-additive gene action. The environment played a significant role in the expression of both the traits. The GCA × environment interaction exhibited greater sensitivity in all cases than the SCA × environment interaction. The varieties Kharchia 65 and Durgapura 65 emerged as desirable general combiners for peduncle length, whereas Pavon and Moncho had high mean performance for flag leaf area. These parents could be used as donors in future breeding to develop a physiologically efficient wheat genotype with high production. The crosses Moncho × Brochis and Durgapura 65 × Raj 821 were the most desirable specific combinations for flag leaf area and Kharchia 65 × Chiroca for both the traits. Desirable transgressive segregants can be expected from these crosses. Diallel selective mating or bi-parental crossing could be useful for the genetic improvement of these physiological traits.

Key words: combining ability, spring wheat, physiological traits, genotype, gene effects

Introduction

The synthesis of a physiologically efficient plant type will help to convert maximum soil and solar energy into the form of biological energy and ultimately more productivity will be expected. In dwarf and semi-dwarf varieties, the internodes are usually very short as compared to tall varieties, while the total number of leaves remains more or less equal. Therefore, due to the shorter internodes, particularly the last internode (peduncle) and the larger number of leaves in a limited culm length, the sunlight does not penetrate the plant properly. However, there is an increase in the total photosynthetic area and ultimately in the end product (Virk and Aulakh, 1975; Jain and Singh, 1976; Prabhu and Sharma, 1984).

Longer peduncles, broader, larger flag leaves (greater flag leaf area) and optimum plant foliage (leaf area index) would be extremely helpful in receiving more sunlight to activate photosynthesis and to transfer the product to the sink more efficiently (Lupton, 1973; Monyo and Whittington, 1973; Ibrahim and Abo Elenein, 1977; Brigga and Aytenfisu, 1980). The morphological part above the flag leaf node contributes about 74% of the total yield in wheat (Mackey, 1982; Mahmood et al., 1991).

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To develop a physiologically efficient and highly productive genotype in wheat, a knowledge of the combining ability of the physiological traits would be useful. Information on the nature of the genetic control of these physiological traits is lacking in wheat. Hence, the present investigation has been undertaken to identify the most suitable genetic material to be used as parents of cultivars in future and to more efficiently finalize the breeding programme. For this, combining ability analysis was performed involving 10 × 10 diallel cross combinations over environments for peduncle length and flag leaf area.

Materials and methods

Ten varieties of bread wheat (*Triticum aestivum* L.), namely Moncho, Pavon, Brochis, Chiroca, HD 2204, Raj 1482, WL 711, Raj 821, Durgapura 65 and Kharchia 65, were crossed in all possible combinations excluding reciprocals. The resulting 45 F_1s were grown to obtain F_2 seeds. The parents, F_1 and F_2 plants were grown in a randomized block design with three replications under early, normal and late sown environments at the Research Farm of the Department of Plant Breeding, Durgapura, Jaipur (Rajasthan). Each plot consisted of single 5 m rows for parental and F_1 plants and ten rows of F_2 plants with a spacing of 30 cm between rows and 15 cm between plants.

Excluding border plants, data were recorded separately on 10 competitive plants per plot in the parents and F_1s and 20 plants per plot in the F_2 progenies under each environment. The peduncle length was measured in centimetres from the top of the flag leaf sheath of the main tiller up to the base of the spike in on selected plants. Flag leaf area (cm^2) was also measured in each environment separately on all the shoots of the selected plants without removing the leaves (Simpson, 1968). Plot means were used for statistical analysis. The data were subjected to analysis of variance for randomized block design for pooled environments (Panse and Sukhatme, 1967). The combining ability estimates over the environments were calculated according to the procedure proposed by Griffing (1956) using method II, Model I.

Results and discussion

Pooled analysis of variance over the environments revealed highly significant differences between the genotypes and sso was for the genotype × environment interactions (Table 1). The pooled analysis of variance for combining ability reflected that both the general combining ability (GCA) and specific combining ability (SCA) mean squares were significant for peduncle length in both the F_1 and F_2 generations. Thus, both kinds of gene effects were important in controlling the inheritance of this trait (Jain and Singh, 1976; Dhindsa, 1982). However, the GCA mean squares were found to be higher than the SCA values in both generations, indicating the preponderance of additive gene effects for peduncle length (Ramirej et al., 1969; Virk and Aulakh, 1975; Prabhu and Sharma, 1984).

For flag leaf area the SCA mean squares were significant in both generations but on account of the high genotype × environment interaction the GCA estimates were not significant for this trait, signifying the importance of non-additive gene effects in controlling the inheritance (Table 1). Flag leaf area was also reported to be under the control of non-additive genes by other authors (Jain and Singh, 1976; Ilyushechenko, 1977; Barriga, 1979; Dhindsa, 1982; Prabhu and Sharma, 1984; Verma, 1988; Joshi, 1997; Singh, 2002).

Table 1
Pooled analysis of variance for physiological traits in spring wheat over three environments

Source	df	Peduncle length		Flag leaf area	
		F_1	F_2	F_1	F_2
		Mean square			
Environments (E)	2	2742.67**	2475.65**	9939.04**	8360.88**
Genotypes (G)	54	156.92**	105.23**	74.38**	58.66**
G × E	108	13.17**	11.39**	39.19**	32.93**
Error	324	3.12	2.89	2.86	4.35
		Combining ability			
GCA	9	264.94**	179.43**	56.30	58.31
SCA	45	9.79**	6.22**	18.49**	11.80**
GCA × E	18	8.99**	8.40**	38.63**	32.47**
SCA × E	90	3.47**	2.88**	7.95**	6.68**
Error	324	1.04	0.96	0.95	1.45

** Significant at the 1% level of probability.

The results further revealed that both the GCA × environment and the SCA × environment interactions were significant in both the generations, indicating the role of environment in influencing the gene effects. However, the relative magnitude of the GCA × environment interactions was higher as compared to the SCA x environment interactions, suggesting greater sensitivity to the environment for GCA than for SCA (Table 1). This is in agreement with many earlier findings (Kumar et al., 1983; Singh et al., 1987; Srivastava et al., 1992; Joshi, 1997; Singh, 2002), though Dasgupta and Mondal (1988) reported greater sensitivity of the SCA × environment interaction. It appears from both the earlier and present findings that the additive genetic variance was less constant from one environment to another than the non-additive genetic variance. The heterozygosity *per se* and the physiological advantages attached to this by virtue of heterosis or enhanced metabolic rates (Sinha and Khanna, 1975) may have contributed to the lower sensitivity of SCA to environmental fluctuations as compared to GCA.

The estimates of GCA effects revealed that the parents Kharchia 65 and Durgapura 65 had good general combining ability for peduncle length in both the F_1 and F_2 generations, while Brochis, Raj 1482, Chiroca, HD 2204 and Pavon were consistently low combiners for this trait (Table 2). Marked differences were observed in the mean performance of the parental lines for flag leaf area (23.0 to 32.7 cm^2), and parents Pavon, Moncho, Kharchia 65 and WL 711 looked promising. However, conclusions regarding their general combining ability could not be drawn, because the GCA estimates were not significant for this trait due to the high GCA × environment interaction (Table 2).

The mean performance of the parents for peduncle length was observed to be related with their GCA effects in the majority of cases. Thus, the selection of lines for breeding programmes could be based on both mean performance and

GCA for peduncle length to accelerate the pace of genetic improvement of this trait in bread wheat. In the case of flag leaf area, mean performance could be used to achieve a tangible improvement in wheat. Considering the mean performance for both the characters together, the variety Kharchia 65 (single gene dwarf) was found to be the best, followed by another single gene dwarf, Moncho, whereas Brochis was observed to be the poorest for these traits.

The analysis of specific combining ability (SCA) effects revealed that out of the 45 crosses in each generation, eleven had significant SCA effects for both the traits studied (Table 3). The results further showed that 9 crosses had positive significant SCA effects for peduncle length in both the generations, whereas 10 crosses in the F_1 and 7 crosses in the F_2 showed positive significant SCA effects for flag leaf area. The inconsistency observed in the SCA effects in these crosses over generations was due to the genotype × environment interaction. Only the cross Kharchia 65 × Chiroca exhibited a consistent, desirable, significant SCA effect, indicating good specific cross combinations for both peduncle length and flag leaf area. The crosses Moncho × Brochis and Durgapura 65 x Raj 821 proved to be desirable specific combiners for flag leaf area in both generations. Other good specific cross combinations were Durgapura 65 × Chiroca, Durgapura 65 × HD 2204 and WL 711 × Durgapura 65 for peduncle length in the F_1, Pavon × Raj 1482, WL 711 × HD 2204 and Chiroca × Raj 821 for peduncle length in the F_2 and Raj 1482 × Raj 821 for flag leaf area in the F2.

Table 2
Estimates of GCA effects and mean performance of the parent varieties pooled over three environments

Parent variety	Peduncle length			Flag leaf area
	Mean	GCA effect		Mean
		F_1	F_2	
Moncho	39.1	0.18	0.47	30.2
Pavon	37.1	–1.27L	–0.47	32.7
Brochis	30.8	–3.67L	–3.28L	28.4
WL 711	39.4	0.84	0.79	29.7
Durgapura 65	40.9	3.11H	1.56H	27.8
Kharchia 65	48.4	5.56H	4.91H	29.9
Chiroca	36.9	–2.26L	–0.08	28.7
HD 2204	33.8	–1.98L	–1.60L	23.0
Raj 1482	33.1	–2.41L	–1.94L	28.5
Raj 821	37.2	–0.10	–0.36	23.0
S. Em. ±		0.47	0.46	
C. D.		1.39	1.34	

Note: For flag leaf area GCA estimates were not significant due to the high G × E interaction. H - High estimates of GCA effects; L - Low estimates of GCA effects

Table 3
Crosses showing significant SCA effects for physiological traits in spring wheat over environments

Cross	Peduncle length		Flag leaf area	
	F_1	F_2	F_1	F_2
Kharchia 65 × Chiroca	3.32 H	2.47 H	5.35 H	5.16 H
Durgapura 65 × Chiroca	2.69 H	1.15	2.86	1.22
Durgapura 65 × HD 2204	2.68 H	1.76	1.02	1.10
WL 711 × Durgapura 65	3.15 H	–0.65	0.84	0.64
Pavon × Raj 1482	0.11	2.69 H	–1.73	–0.99
WL 711 × HD 2204	–1.18	2.77 H	2.28	–0.43
Chiroca × Raj 821	0.30	2.19 H	2.12	–1.21
Moncho × Brochis	1.01	1.38	3.66 H	4.85 H
Durgapura 65 × Raj 821	2.17	0.38	4.32 H	3.03 H
Raj 1482 × Raj 821	0.77	1.80	2.02	3.24 H
Moncho × Raj 1482	–0.45	–0.39	3.43 H	–0.85
S. Em. ±	0.89	0.81	1.35	1.24
C. D.	2.73	2.49	4.14	3.79

H - High estimates of GCA effects

In order to utilize the crosses efficiently, the *inter se* crossing of F_1s in all possible combinations or multiple parent input into a central gene pool will lead to the discovery of better recombinants and may also help in breaking the genetic barriers, if any (Jensen, 1970).

It is noteworthy that the cross Kharchia 65 × Chiroca, which showed a high SCA effect for both peduncle length and flag leaf area, was a combination of Indian × exotic types (indigenous × extraneous germplasm). Other crosses also showed this trend for both the traits studied. This emphasizes the need for combining two diverse germplasms to create maximum genetic variability, which is a prime requirement and would alone help to expand the limits of envisaged progress through selection in any successful breeding programme for genetic improvement of these traits in bread wheat.

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IMPROVEMENT OF COMMERCIAL MAIZE LINES THROUGH THE INTEGRATION OF GENES AND GENE COMBINATIONS FROM ELITE LINES

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Between 1980 and 2000 two parallel breeding experiments were carried out to examine the effect of pedigree, backcrossing to the elite line, early testing, visual selection and late testing on the development of inbred maize lines with commercial value.

In both series of experiments the standard was a hybrid between the line used as tester and the line chosen for improvement (HMv 9). Early testing was carried out using testers F 2 and HMv 23. In agreement with the literature, the frequency distribution indicated that at least half the families gave a higher yield on both testers than the original line. In the case of grain moisture and stalk strength, the derivatives of the individual populations behaved differently on each tester, suggesting the presence of a tester × donor interaction. On the F 2 tester no families were found which yielded significantly better, while also having significantly lower grain moisture and/or significantly better stalk strength. On tester HMv 23 one family was found which yielded significantly more than the standard while also having significantly lower grain moisture. The final evaluation demonstrated that 6 lines with commercial value were developed in the two breeding experiments; these were used in the breeding of 12 registered hybrids. The performance of the source populations chosen for use in line development was found to be extremely important in the development of lines with commercial value. By comparison the methods used for line development and the testing conditions were of secondary importance and were found to have no significant influence on the tested populations. It was concluded that in breeding programmes aimed at developing commercial lines, even greater attention should be paid to the performance of the source populations. In most cases, due to the small number of families/populations, the fixing of traits during inbreding takes place in a random manner, and selection has little modifying effect as the generations become increasingly homozygous. For the above reasons it is wiser and more economical to choose the simple, cheap line development method.

Key words: maize, maize breeding, inbred line development

Introduction

According to Shull (1908, 1909) the purpose of hybrid maize breeding is to develop and identify the best possible hybrids. This statement is still true, with the reservation that hybrids better than those available today can only be bred using lines which are also better than the present ones. On the other hand, as far back as the 1940s, when previously sampled open-pollinating varieties were resampled, it became clear that better lines could only be selected from sources better than those currently available. It is thus obvious that sources must constantly be improved.

After much debate, two fundamental concepts were developed. Jenkins (1935, 1940), Sprague and Tatum (1942), Hull (1945), Comstock et al. (1949) and other authors used the available elite lines, varieties and populations as components in the development of new synthetic varieties, variety populations and gene pools. It was thought that if these populations, which had a broad genetic background, were improved from one cycle to the next, lines with better breeding value would gradually appear with greater frequency (Sprague and Eberhart, 1977).

Hayes and Johnson (1939) and Johnson and Hayes (1940) sought for a solution in another direction. They began to use the pedigree method, known and successfully used in animal breeding for thousands of years, to develop maize lines with increasingly good breeding value.

Russell and Teich (1967), El-Lankany and Russell (1971) and Russell and Machado (1978) made further important discoveries. The effect of various types of visual selection, disruptive selection for combining ability and various stress factors on the value of the new lines was studied in multifactorial breeding trials. Lonnquist (1974), Hallauer (1978) and more especially Lamkey et al. (1995), Hadi (1993, 2003b) and Hadi et al. (2002) examined the effect of plants traits and genetic linkage on the breeding value of the lines obtained. Dudley (1982, 1984a, b) elaborated a pairing model and the relevant statistics as a guideline for the recognition of favourable new or more efficient genes and gene combinations not present in commercial lines, but available in donors, and for their integration into commercial lines.

Data from the literature and breeding experience (Gerdes et al., 1994) indicate that with the exception of a single synthetic variety, BSSS (B 73, B 84), commercial maize lines with good breeding value have not been produced in recurrent selection programmes (Hadi, 2003a, b). The theory presented by Sprague and Eberhart (1977) is of little assistance as regards the factors limiting an increase in grain yield during population improvement. Breeding experience suggests that the selection possibilities available for population improvement are not infinite. The probable limits for any trait are the extreme values of phenotypic frequency distribution. In the case of grain yield the extreme value for a population with average variability is the mean value ± 120–160% ($-a<m<+a$). If we wish to expand the extreme values, crosses must be made with suitable partners (Hadi, 2003a). Several variants of pedigree breeding have been successfully employed for the development of better lines (Troyer, 1999; Dudley 1982; 1984a, b; 1987; 1988; Zanoni and Dudley, 1989; Misevic, 1989a, b, c). The pedigree method also corresponds well with genetic linkage. Selection for the required phenotype will help to preserve the genes and gene combinations proved by long cycles of breeding to be effective in increasing yield potential. Genetic linkage allows new genes and gene combinations to be incorporated and integrated into the traits it is desired to preserve (Hadi 2003b). The limitations of the pedigree method also became obvious in the 1940s, since it was found that

the method gradually integrated smaller sources of heterosis into larger ones, thus limiting the magnitude of the hybrid effect, an increase in which is the basic aim. In order to retard this process the existing lines were divided into heterotic groups A and B and an agreement was reached on how pedigrees should be compiled. Integration continues, however. Commercial lines with mixed Lancaster/Reid Y. D., Lancaster/Flint or Reid Y. D./Flint backgrounds can now be found, which are not only of limited use, but also prevent new sources from being created for the maize breeders of the future.

The testing of various concepts and methods for line development frequently takes place in the early inbred generations, so full information is not obtained either on the final value of lines developed under different conditions with different methods or on the frequency with which commercial lines are produced. The value of the initial pedigree, and the effectiveness, success and profitability of the selection conditions and methods are, however, determined primarily by the extent to which they enable commercial lines to be developed.

An attempt was made to evaluate and compare the various concepts, sources, breeding conditions and methods on the basis of breeding experiments carried out over a 20-year period between 1980 and 2000. Answers were sought to the following questions:

1. Can favourable new genes or gene combinations present in the donor but absent from the commercial lines be most efficiently incorporated with or without backcrossing?

2. When elite pedigrees are used, what are the advantages of early testing and continual testing throughout the inbreeding process compared with conventional line development based on visual selection and late testing?

Materials and methods

When the experiment was set up it was known that the closed pedigree maize line HMv 9 had resulted in commercial hybrids when crossed with the closed pedigree lines HMv 1, HMv 2, HMv 3 and HMv 4, while it also combined extremely well with the closed pedigree lines HMv 5, HMv 6, HMv 7 and HMv 8, and with lines HMv 23 and F 2. The listed lines also combine well with each other, indicating that they are not closely related to each other, but these crosses did not lead to the creation of commercial hybrids. The basic concept was that lines HMv 1, HMv 2, HMv 3 and HMv 4 might contribute genes and gene combinations missing from HMv 9 in order to improve this line, while the newly developed lines related to HMv 9 could be crossed with the other listed lines, or with variants of these, to produce commercial hybrids.

Two series of experiments were set up in 1981:

Experiment I. Lines HMv 1, HMv 2, HMv 3 and HMv 4 were each crossed with line HMv 9. Of all these lines, HMv 9 had the best breeding value. Consequently, following the suggestion made by Dudley (1987), the hybrids were backcrossed to line HMv 9 on one occasion in 1982. The BC_1 populations were sibbed in 1983, then in 1984 forty S_0 plants from each of the BC_1 F_2 populations were self-fertilised in plots containing 40 × 40 plants, after which spare pollen from the marked plants was crossed with the F 2 line tester. The yield from the marked plants was harvested separately and the BC_1S_1 families were sown on 40-plant plots in 1985. These plants were again self-fertilised and test crosses were made on tester HMv 23 using mixed pollen from

the plants of each family. Also in 1985 the F 2 tester × BC_1S_0 crosses were sown at three locations (Martonvásár, Iregszemcse, Baja) with two replications, using the F 2 × HMv 9 hybrid as the standard. In 1986 the HMv 23 tester × BC_1S_1 families were tested at the same locations, using the hybrid HMv 23 × HMv 9 as the standard. All the BC_1S_2 families were sown and self-fertilised in 1986, and at the end of the year the results of the 1985 and 1986 test crosses were evaluated. The results were not averaged over the testers, because seed was not obtained for every combination. In 1987, after evaluation and selection, all the families that gave more yield than the standards on each of two testers and whose grain moisture content and/or stalk lodging were not higher than those of the standards on either tester, were sown and self-fertilised. All the families sown were crossed with HMv 5 in 1987, with HMv 6 in 1988, with HMv 7 in 1989 and with HMv 8 in 1990, and the hybrids were included in performance trials the year after the test cross. Among the S_3 families sown in 1987, some were rejected due to susceptibility to disease (ear and grain fusariosis, viral diseases) or problems in seed production.

Experiment II. In the second series of experiments the F_1 hybrids developed in 1981 (HMv 1 × HMv 9, HMv 3 × HMv 9, HMv 4 × HMv 9) were sibbed in 1982. The following year forty S_0 plants from each population were self-fertilised in plots containing 40 × 40 plants. In 1984 forty S_1 families from each population were sown and self-fertilised. Visual selection was carried out in the usual manner, without testing, primarily for plant diseases and for traits important in the seed industry. Inbreeding was continued until homozygozity was reached. In 1987 the families retained were crossed with lines HMv 5, HMv 6, HMv 7 and HMv 8, as described in Experiment I, and the hybrids were evaluated in performance trials the year after crossing. In this experiment no families were rejected due to poor combining ability.

The plant density was 80,000 plants/ha in all the performance trials and 100,000 plants/ha in the nursery. With a total annual rainfall of 500 mm/year and uneven rainfall distribution, this represents substantial, but not excessive stress. In Experiments I and II the recommendations of Russell and Machado (1978) and Maita and Coors (1996) were followed during inbreeding in an attempt to select for two-eared plants. The performance trials were evaluated using analysis of variance and the frequency distribution around the standard, as suggested by Misevic (1989a). In each year the standards used were always the hybrid of the given tester and the hybrid HMv 9. Among the plant traits, grain yield, grain moisture at harvest and lodging data were processed.

Results

Analysis of variance showed significant ($P<0.01$) differences for grain yield and grain moisture in 1985 and 1986, and for stalk strength in 1985. In 1986 the stalk strength error in crosses made on the tester HMv 23 was small (0.0–1.2%) at all three locations. The analysis of variance was not significant, probably due to data collection errors, so this was not included in the evaluation. A high level of significance ($P<0.01$) was obtained for the location and the hybrid in both years in the case of grain yield and grain moisture. The location × hybrid interaction, however, was not significant in either year for either trait. This could be explained by the fact that the order of the hybrids was similar despite the growing site differences. This result was not unexpected, as the hybrids were closely related to each other.

For the hybrids developed on the F 2 tester in 1985, the frequency distribution around the F 2 × HMv 9 standard in the case of grain yield, grain moisture at harvest and stalk strength is presented in Table 1.

Table 1
Frequency distribution of grain yield, grain moisture and stalk strength in BC_1S_0 lines tested on tester F 2, in comparison with the standard F 2 × HMv 9

Hybrid	1	2		3		4		5	
		No.	%	No.	%	No.	%	No.	%
				Grain yield					
F2×(HMv1×HMv9²) S_0	28	1	3.6	19	67.9	8	28.6	–	–
F2×(HMv2×HMv9²) S_0	32	1	3.1	22	68.8	9	28.1	–	–
F2×(HMv3×HMv9²) S_0	25	0	0.0	17	68.0	8	32.0	–	–
F2×(HMv4×HMv9²) S_0	37	0	0.0	1	2.7	36	97.3	–	–
				Grain moisture					
F2×(HMv1×HMv9²) S_0	28	–	–	20	71.4	8	28.6	0	0.0
F2×(HMv2×HMv9²) S_0	32	–	–	23	71.9	9	28.1	0	0.0
F2×(HMv3×HMv9²) S_0	25	–	–	16	64.0	9	36.0	0	0.0
F2×(HMv4×HMv9²) S_0	37	–	–	1	2.7	18	48.6	18	48.6
				Stalk strength					
F2×(HMv1×HMv9²) S_0	28	–	–	22	78.6	6	21.4	0	0.0
F2×(HMv2×HMv9²) S_0	32	–	–	27	84.4	5	15.6	0	0.0
F2×(HMv3×HMv9²) S_0	25	–	–	23	92.0	2	8.0	0	0.0
F2×(HMv4×HMv9²) S_0	37	–	–	24	64.9	13	35.1	0	0.0

1: No. of BC_1S_0 lines tested; 2: BC_1S_0 lines with higher values than the standard at the P<5% level; 3: BC_1S_0 lines with higher values than the standard numerically; 4: BC_1S_0 lines with lower values than the standard numerically; 5: BC_1S_0 lines with lower values than the standard at the P<5% level

With respect to grain yield, the (HMv 1 × HMv 9²) S_0 and (HMv 2 × HMv 9²) S_0 families both contained one family which yielded significantly more than the standard, while 67.9%, 68.8%, 68% and 2.7% of the families yielded numerically more than the standard among the (HMv 1 × HMv 9²) S_0, (HMv 2 × HMv 9²) S_0, (HMv 3 × HMv 9²) S_0 and (HMv 4 × HMv 9²) S_0 families. A similar distribution around the standard was observed for grain moisture and stalk strength in these families. It is favourable that for three of the four populations more than 50% of the families yielded more than the standard. It is unfortunate, however, that the grain moisture of these same families was also higher than that of the standard. The only exception was (HMv 4 × HMv 9²) S_0, where only one family had a grain moisture greater than that of the standard. This may be due to the fact that the families selected from the (HMv 1 × HMv 9²) S_0, (HMv 2 × HMv 9²) S_0 and (HMv 3 × HMv 9²) S_0 populations had a similar or somewhat longer vegetation period than line HMv 9, while that of the families selected from the (HMv 4 × HMv 9²) S_0 population was shorter. The frequency distribution figures for stalk strength suggest that, as hybrids from the families of all four populations lodged to a greater extent than the standard and no families with better stalk strength than the standard were found, the donors provided no genes contributing to an improvement in stalk strength.

The frequency distribution of test crosses on HMv 23 around the HMv 23 × HMv 9 standard is presented in Table 2.

Table 2
Frequency distribution of grain yield and grain moisture in BC_1S_1 lines tested on tester HMv 23, in comparison with the standard HMv 23 × HMv 9

Hybrid	1	2		3		4		5	
		No.	%	No.	%	No.	%	No.	%
				Grain yield					
HMv23×(HMv1×HMv9²) S_1	28	1	3.6	9	32.1	18	64.3	–	–
HMv23×(HMv2×HMv9²) S_1	30	2	6.7	21	70.0	7	23.3	–	–
HMv23×(HMv3×HMv9²) S_1	26	1	3.8	11	44.3	14	53.8	–	–
HMv23×(HMv4×HMv9²) S_1	36	0	0.0	1	2.8	35	97.2	–	–
				Grain moisture					
HMv23×(HMv1×HMv9²) S_1	28	–	–	7	25.0	11	39.3	10	35.7
HMv23×(HMv2×HMv9²) S_1	30	–	–	4	13.3	10	33.3	16	53.3
HMv23×(HMv3×HMv9²) S_1	26	–	–	11	42.3	12	46.1	3	11.5
HMv23×(HMv4×HMv9²) S_1	36	–	–	2	5.6	0	0.0	34	94.4

1: No. of BC_1S_1 lines tested; 2: BC_1S_1 lines with higher values than the standard at the P<5% level; 3: BC_1S_1 lines with higher values than the standard numerically; 4: BC_1S_1 lines with lower values than the standard numerically; 5: BC_1S_1 lines with lower values than the standard at the P<5% level

Families yielding significantly more than the HMv 23 × HMv 9 standard were found with a frequency of 3.6%, 6.7% and 3.8% among the (HMv 1 × HMv 9²) S_1, (HMv 2 × HMv 9²) S_1 and (HMv 3 × HMv 9²) S_1 families. In the first three populations the distribution around the standard exhibited greater deviation on tester HMv 23 than on F 2. This was also true in the case of grain moisture. It was interesting to note that 35.7%, 53.3%, 11.5% and 94.4% of the test crosses for the families of the various populations listed above could be harvested with significantly lower grain moisture than their respective standards. As a combined analysis for each family over the two years was not carried out, it is difficult to define the reasons for this, but it is thought that it could be due to the tester × donor interaction. (Hybrids from line F 2 flowered early and had high grain moisture, while those originating from HMv 23 flowered late and had low grain moisture at harvest. Hybrids of HMv 1, HMv 2 and HMv 3 had an intermediate silking date and above-average grain moisture, while those of HMv 4 silked early and had extremely low grain moisture at harvest.)

Table 3 illustrates the mean contribution of donors HMv 1, HMv 2, HMv 3 and HMv 4 to the improvement of HMv 9. Depending on the tester, the populations were compared to the standards F 2 × HMv 9 and HMv 23 × HMv 9. It can be seen that the mean of the test crosses only exceeded that of the standards for HMv 1 and HMv 2 on tester F 2 and for HMv 2 on tester HMv 23, giving mean yield increments of +0.19, +0.29 and +0.17 t/ha, respectively. In the case of grain moisture, the values recorded for families from the first three populations on tester F 2 were generally +0.58%, + 0.27% and +0.39% higher on average than that of the F 2 × HMv 9 standard, while on tester HMv 23 the families of all three populations had a lower mean grain moisture than the (HMv 23 × HMv 9) standard. The (HMv 4 × HMv 9²) families had lower mean values than the standards on both testers. As regards stalk strength, the families of all four populations exhibited higher means on tester F 2 than the F 2 × HMv 9 standard. No data are available for tester HMv 23.

Table 3
Mean contribution of donors HMv 1, HMv 2, HMv 3 and HMv 4 to improvements in the grain yield, grain moisture and stalk strength of line HMv 9 when tested on the early-maturing line F 2 and the late-maturing line HMv 23, compared with the standards F 2 × HMv 9 and HMv 23 × HMv 9, respectively

Hybrid	1	2	3	4
F2×(HMv1×HMv9^2) S_0	28	+0.19	+0.58	+1.64
F2×(HMv2×HMv9^2) S_0	32	+0.29	+0.27	+1.93
F2×(HMv3×HMv9^2) S_0	25	–0.26	+0.39	+2.19
F2×(HMv4×HMv9^2) S_0	37	–0.74	–1.37	+1.16
HMv23×(HMv1×HMv9^2) S_1	28	–0.36	–1.04	–
HMv23×(HMv2×HMv9^2) S_1	30	+0.17	–1.37	–
HMv23×(HMv3×HMv9^2) S_1	26	–0.12	–0.12	–
HMv23×(HMv4×HMv9^2) S_1	36	–1.50	–3.02	–

1: No. of BC_1S_0 and BC_1S_1 families tested; 2: Grain yield of the test cross (X_{TC}–X_C) (t/ha); 3: Grain moisture of the test cross at harvest (X_{TC}–X_C) (%); 4: Lodging of the test cross (X_{TC}–X_C) (%)

The combined frequency of grain yield and grain moisture around the standards is summarised in Table 4. Positive changes for two plant traits were only observed in combination for one family, (HMv 3 × HMv 9^2) S_1, on tester HMv 23, equivalent to a frequency of 3.8%, and for none on tester F 2. This family gave significantly higher yield with significantly lower grain moisture, but was rejected in later stages of inbreeding due to its great susceptibility to fusarium ear rot. No examples were found of positive changes for all three traits.

The final evaluation was carried out on four lines from population (HMv 1 × HMv 9^2), four from (HMv 2 × HMv 9^2), one from (HMv 3 × HMv 9^2) and one from (HMv 4 × HMv 9^2) in Experiment I and on four lines from population (HMv 1 × HMv 9), two from (HMv 2 × HMv 9), three from (HMv 3 × HMv 9) and two from (HMv 4 × HMv 9) in Experiment II (Table 5).

No lines with commercial value were developed from population (HMv 1 × HMv 9^2), while a single commercial line was produced from each of the pedigrees (HMv 2 × HMv 9^2), (HMv 3 × HMv 9^2) and (HMv 4 × HMv 9^2), each of which resulted in a single commercial line, which were used in breeding silage maize hybrids with a short vegetation period. Two commercial lines were developed from (HMv 1 × HMv 9) and one from (HMv 4 × HMv 9) after visual selection and development without backcrossing, while none were found for (HMv 2 × HMv 9) or (HMv 3 × HMv 9).

It is interesting to note that although the (HMv 1 × HMv 9) population was not the most promising on the basis of the data in Tables 1, 2 and 3, the two lines selected from this pedigree proved to have the best breeding value. Four hybrids bred using each of these lines were still cultivated ten years after registration. It would appear that the donors may contribute both favourable and unfavourable genes and gene combinations to the improved lines. In the case of small populations there is little possibility of transferring all the favourable genes possessed by the donor into the improved line. For this reason, an apparently less valuable donor may contribute as much or sometimes more to the improvement of an elite line, than one expected to have greater value, but which is not sufficiently exploited.

Table 4
Contribution of donors HMv 1, HMv 2, HMv 3 and HMv 4 to improvements in the grain yield and grain moisture of line HMv 9 compared with the standard HMv 23 × HMv 9

Hybrid	No. of BC_1S_1 families tested	Total frequency of BC_1S_1 lines with a significantly higher yield and significantly lower grain moisture than the standard (P<5%)	
		No.	(%)
HMv23×(HMv1×HMv9^2) S_1	28	0	0.0
HMv23×(HMv2×HMv9^2) S_1	30	0	0.0
HMv23×(HMv3×HMv9^2) S_1	26	1	3.8
HMv23×(HMv4×HMv9^2) S_1	36	0	0.0

Table 5
Commercial lines developed by visual selection at high plant density with and without backcrossing and testing

Pedigree	1	2	3	4	5	6	7	8
Experiment I								
(HMv1×HMv9^2)	7	4	–					
(HMv2×HMv9^2)	9	4	1	1	1994	Silage	4	F.
(HMv3×HMv9^2)	5	1	1	1	1993	Silage	6	G., U., H.
(HMv4×HMv9^2)	6	1	1	1	1995	Silage	3	B., F.
Experiment II								
(HMv1×HMv9)	–	4	2	4+4	1993–1994	Grain	<10	H., R., U., S.
(HMv2×HMv9)	–	2	–	–	–	–	–	–
(HMv3×HMv9)	–	3	–	–	–	–	–	–
(HMv4×HMv9)	–	2	1	1	1992	Silage	5	R.
Total		21	6	12	–	–	–	–

1: No. of families selected in early generations; 2: No. of lines developed; 3: No. of lines with commercial value; 4: No. of registered hybrids; 5: Year of registration; 6: Type of utilisation; 7: Lifespan of the hybrids (years); 8: Countries in which registered; F: France; G: Germany; U: Ukraine; H: Hungary; B: Belgium, R: Russia; S: Slovakia

Discussion

The results confirm those of Misevic (1989c), who found that the initial pedigrees had a fundamental influence on whether lines with commercial value could be selected. When designing the initial pedigree, attention must be paid to which heterosis source the components were derived from, in an effort to prevent genetic mixing between the various heterosis sources. Although the method reported by Dudley (1984a) gives no guidelines in this respect, in practice breeders should use lines with a similar genetic background, which may be able to provide favourable genes and gene combinations without the danger of negative changes due to recombination.

A comparison of the results obtained in Experiments I and II does not make it clear whether it is necessary to backcross to the better parent, since

commercial lines were obtained with the same frequency from both experiments. The results of early testing only exhibited significantly higher yield combined with significantly lower grain moisture for one family on one tester. It would appear that the 40 BC_1S_1 families (altogether 160 in the present case) used in breeding practice give too few positive recombinations for the transfer of all the favourable traits, while the 40 plants/family used in inbreeding generations S_1–S_6 was either too few, or fixation was too rapid, to have any fundamental influence on the selection of positive recombinations. It is also possible that the contribution of the selected donors to a real improvement in line HMv 9 was estimated too optimistically.

The results confirm those reported by Russell and Teich (1967), El-Lankany and Russell (1971) and Russell and Machado (1978), who found that the selection conditions and breeding methods applied during inbreeding had no major effect on the value of the inbred lines, although the distinction between valuable and less valuable lines could be enhanced by various conditions and methods.

The authors are in agreement with Dudley (1982) and Misevic (1989a) as regards the selection of donor partners. For three of the four pedigrees, more than 50% of the 40 BC_1S_1 lines from each pedigree with a given tester yielded numerically more than the relevant standard, while lines selected from one pedigree were earlier. The predictions made by Dudley (1987) and Misevic (1989a) were found to be too optimistic, over-estimating what could be achieved. It would be more realistic to aim at developing lines with similar value, having a tendency for their hybrids to yield more with the same or lower grain moisture and the same or better stalk strength.

It proved impossible to develop lines significantly better than the original HMv 9 line, but the results achieved in Experiments I and II indicated that lines with commercial value could be developed from all the pedigrees. The twelve commercial hybrids developed using a total of six lines, three from Experiment I (1.88%) and three from Experiment II (1.88%), had a variety of phenotypes, vegetation periods, adaptability and end uses, while often being more resistant to plant diseases. However, the suitability of the new lines for economical seed production was generally worse than that of the original HMv 9 line.

The frequency with which commercial lines were selected (6 from a total of 320 S_1 families) was higher than that calculated by Hallauer and Miranda (1981), who stated that only 38 lines from a total of 360,000 S_2–S_3 lines tested at American universities between 1940 and 1975 had a share of more than 0.1% in the seed industry. It was also higher than that previously achieved by the authors (Hadi, 2003a), who were unable to select lines with commercial value when testing 12,500 S_1 families developed from open-pollinated and synthetic varieties. Comparing the current results with previous results it can be seen that the value of the commercial lines selected from various sources was determined primarily by the performance of the source pedigree. This indicates that even

greater attention should be paid to sources and pedigrees. Continuous selection for combining ability has little effect on the breeding value due to the small population size used for inbreeding and the rapid fixation of traits. Its major role is in distinguishing the value of the lines. In the course of inbreeding it is more expedient and economical to select for disease resistance and traits ensuring better seed production using simple, cheap phenotypic selection.

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EFFECT OF WEED CONTROL TREATMENTS AND HILL-SPACING ON SOYBEAN AND ASSOCIATED WEEDS

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Two field experiments were carried out at the Experimental Farm of Assiut University, during the 2000 and 2001 summer seasons, to study the effect of three hill spacings (5, 10 and 15 cm) and six weed control treatments on the associated weeds, plant growth, yield and quality of soybean. The weed control treatments were carried out with trifluralin, linuron, pendimethalin, bentazon and hand hoeing, with an unweeded treatment as the control.

All the weed control treatments exerted a significant influence on the dry weight of weeds. Hand hoeing and pendimethalin treatment significantly decreased the dry weight of dicot and monocot weeds as compared to the unweeded treatment. Hand hoeing gave the lowest value of the dry weight of total weeds and the highest efficiency percentage. In general, the significantly lowest dry weight of total weeds was recorded for densely sown soybeans.

The weed control treatments exerted a significant influence on all the characters under study (plant height, weight of pods and seeds/plant, number of plants at harvest, seed yield/ha). The height of the first pod was lowered by hand hoeing and the application of pendimethalin increased the number of pods/plant, while the highest values of number of branches/plant, seed index, oil and protein contents were obtained after linuron application.

Wider spacing produced higher values for the number of pods and branches/plant, weight of pods and seeds/plant, seed index and protein content and lowered the height of the first pod as compared to plants sown at closer spacing. The latter produced the highest values of number of plants/ha, seed yield/ha and oil content at harvest.

The first order interaction exerted a significant influence on all the characters studied, the highest seed yield/ha (2728.6 kg) being obtained from sowing plants at 5 cm combined with hand hoeing.

Key words: soybean, associated weeds, weed control, herbicides

Introduction

Soybean is an important oil-yielding legume, bearing seeds containing about 20% oil and 40% protein. It is also used in the formulation of concentrated animal feed. Egypt is facing a severe shortage of both oil and animal feed. Thus, an increase in the productivity of soybean is a strategic goal of the Egyptian Agricultural System. Weeds constitute a serious economic problem to crop production. The soybean yield is more significantly decreased by weed competition for nutrients, water and light than by any other factor. Therefore, weed control is essential, especially during the early development of soybean (Muniyappa et al., 1986). At the present time there is a great shortage of hand

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labour, combined with a rise in the wage scale, making the use of chemical weed control very necessary to decrease costs and to increase the production of soybean. Jain and Acharya (1985) found that pendimethalin gave good control of grasses in soybean fields, while linuron and pendimethalin gave effective control of monocots in soybean. On the other hand, Lakres et al. (1987) reported that hand hoeing gave more effective weed control than any herbicide, and resulted in the greatest yield. Gaweesh (1987) mentioned that the application of hand hoeing, pendimethalin or linuron increased the pod and seed yield/plant, 100-seed weight, seed and straw yield/ha and seed protein yield. Zaki et al. (1993) reported that the application of bentazone for weed control in soybean increased growth and yield. Dubey (1998) concluded that hand-weeding increased the seed yield of soybean by reducing the weed population and weed dry matter significantly. Joshi and Billore (1998) revealed that two hand-weedings 30 and 45 days after sowing were effective in controlling weeds in soybean. Hassanein et al. (2000) reported that the application of pendimethalin, oxyfluorfen or linuron in combination with bentazone was effective and comparable to hand-weeding from the point of view of weed control and yield. Mandloi et al. (2000) indicated that hand-weeding 30 and 45 days after sowing produced the highest seed yield of soybean with the lowest weed dry matter. Nayak et al. (2002) showed that the weed population and weed dry matter were the lowest and the weed control efficiency highest in weed-free treatments, followed by two hand-weedings and pendimethalin. The highest seed yield was recorded in the weed-free treatment, which was at par with two hand-weedings and pendimethalin. Chirita et al. (1998) revealed that the best control of annual weeds (80% control) was achieved with flumetsulam (50 g/ha) in combination with Treflan (trifluralin) (at 1.8 litres/ha) applied before sowing. Jain et al. (2000) showed that the pre-emergence application of alachlor and pendimethalin was comparable with two hand-weedings (20-30 days after sowing) in reducing the weed density and weed biomass and in increasing the weed control efficiency as well as the number and weight of pods/plant of soybean. El-Quesni et al. (2002) showed that bentazone, oxyfluorfen, flowzifop and pendimethalin treatments, as well as hand hoeing, showed a significant improvement in yield and its components. The superiority of the hand hoeing treatment was significant as regards the yield and yield components of soybean. Hand hoeing and pendimethalin significantly decreased the total weeds in soybean fields followed by fluazifop-butyl, oxyfluorfen and bentazone compared with that of the unweeded control.

Duncan (1986) stated that a greater seed yield could be obtained in soybean due to greater light interception and dry matter production before seed initiation. Thus, factors that control plant size, such as narrow-row spacing, tend to increase the yield. Plant spacing greatly affects the leaf area light interception (Wells, 1991). Narrow-row spacing results in a sufficient leaf area index (LAI) leading to maximal light interception during seed formation. Parvez et al. (1989)

reported that an increase in plant density in soybean increased total seed yield, height of the first pod and 100-seed weight, and decreased the number of pods and branches/plant. Dubey (1998) revealed that a closer row spacing of 22.5 cm reduced the weed population and dry matter. Ball et al. (2000) reported that increasing the population reduced the yield per plant but increased the yield per unit area. In high populations, the plants maintained individual seed mass by reducing the proportion of shell mass per pod. The reduction in yield caused by low population density was due to the low seed number. Pires et al. (2000) showed that a density of 40 plants/m^2 gave a greater potential yield than 30 plants/m^2Ennin and Clegg (2001) concluded that the soybean yield was highest at populations of $\geq$129,000 plants/ha.. A row spacing of 20 cm gave a higher yield than 40 cm. Andrade et al. (2002) reported that the seed yield increase in response to narrow rows was closely related to the improvement in light interception during the critical period of seed set.

This investigation was carried out to study the effect of weed control treatments and hill-spacing on soybean productivity and associated weeds under middle Egypt conditions.

Materials and methods

Two field experiments were conducted at the Experimental Station Farm of Assiut University during the 2000 and 2001 summer seasons, to study the effect of weed control treatments and hill-spacing on growth, yield, yield components, chemical composition and associated weeds of soybean. Soybean seeds of the Clark variety were treated with *Rhizobium japonicum* before sowing. The seeds were sown on 27th April 2000 and 1st May 2001. The plot area was 10.5 m^2 (3×3.5 m) and each plot consist of 6 rows (60 cm apart and 3 m along). The physical and chemical analysis of a representative soil sample is shown in Table 1.

Table 1
Physical and chemical analysis of a representative soil sample

Character	Value	
	2000	2001
Particle distribution		
Clay %	48.66	48.89
Silt %	28.56	28.55
Sand %	22.78	22.56
Textural grade	Clayey	Clayey
Field capacity %	42.0	41.0
Ec mmhos/cm (1:1)	0.72	0.76
pH (1:1 suspension)	7.40	7.5
Organic matter %	1.84	1.45
Total nitrogen %	0.72	0.80
$CaCO_3$ %	4.00	3.70
Available phosphorus (ppm)	9.10	9.10

A split-plot design with four replications was used, where the treatments were arranged as herbicides in sub-plots and hill-spacing in whole plots.

The weed control treatments were as follows:

(1) Trifluralin 48% W/V (Treflan), at 2.38 l/ha, applied pre-sowing

(2) Linuron 50% WP (Afalon), at 2.38 kg/ha, applied as pre-emergent treatment.

(3) Pendimethalin 50% EC (Stomp), at 4.05 l/ha, applied as pre-emergent treatment.

(4) Bentazone 48% EC (Basagran), at 1142.4 g/ha, as post-emergent treatment.

(5) Hand-hoeing treatment (twice), 20 and 40 days after sowing.

(6) Unweeded (control).

The hill-spacing treatments were 5, 10 and 15 cm between hills. Two plants were left per hill after complete germination.

Nitrogen fertilizer, in the form of ammonium nitrate (33.5% N), was applied at a rate of 142.8 kg/ha before the first irrigation. Superphosphate (15.5% P_2O_5) was applied before sowing at a rate of 357 kg/ha.

Weed survey

Weeds were hand pulled from one square metre in each plot 45 and 75 days after sowing and classified as monocot, dicot and total weeds. The dry weight of each group was recorded as g/m^2. The herbicide efficiency was estimated from the total weed dry weight with the following formula (Mani et al., 1973):

$$EC\% = \frac{Pc - Pt}{Pc} \times 100$$

where: EC = efficiency coefficient, Pc = average dry weight of weeds per m^2 in the unweeded treatment, Pt = average dry weight of weeds per m^2 in the treated plots.

At harvest a random sample of five plants was taken from each plot to record the following: plant height (cm), number of branches/plant, the node bearing the first pod on the main stem, number and weight of pods/plant and seeds/plant (g) and 100-seed weight (g). The number of plants/ha was calculated at harvest, while the seed yield/ha was estimated on a yield/plot basis.

The nitrogen percentage was determined as outlined by A.O.A.C. (1980) and used to calculate the protein content of the seeds using a factor of 6.25. The oil percentage in the seeds was determined according to A.O.A.C. (1980).

Combined analysis of data for the two growing seasons was performed according to Gomez and Gomez (1984). Before the statistical analysis the weed dry weight was transformed to log numbers to obtain normal distribution (Ewrin et al., 1996). The means were compared at the $LSD_{5\%}$ level. The Bartlett test of homogeneity for error indicated that the variance of the data was insignificant in both seasons, thus allowing the combined analysis to be carried out.

Results and discussion

Effect of weed control treatments on dry weight of weeds

The experimental field was infested with various weed species, including both broad-leaved weeds and grasses, the former outnumbering the latter. The dominant broad-leaved weeds were *Convolvulus arvensis* L., *Portulaca oleracea* L., *Chenopodium* spp. and *Xanthium strumarium* L. The most frequent grass weeds were *Cyperus rotandus* L., *Cynodon dactylon* L. and *Echinochloa colonum* L.

The combined data in Table 2 revealed that the weed control treatments significantly reduced the dry weight of grass weeds (g/m^2) as compared with the unweeded treatment. The reduction in the dry weight of grass weeds (g/m^2) due to the application of pendimethalin and trifluralin was 98.4% and 96.5% at 45 days and 99.39% and 92.11% at 75 days, respectively, as compared with the unweeded treatment. Jain and Acharya (1985) also found that pendimethalin gave good control of grasses in soybean fields.

The weed control treatments significantly affected the dry weight of the broad-leaved weeds in both samples. The reductions obtained from the application of bentazone, linuron and hoeing were 95.6%, 83.6% and 94.2% on the 45th day and 98.78%, 91.17% and 99.50% at 75 DAS, respectively, as compared with the unweeded treatment.

The effect of weed control treatments on the dry weight of total weeds was significant on both dates (45 and 75 DAS). As regards weed biomass accumulation, hand-weeding was better than the other treatments in reducing the dry weight of total weeds. Similar results were reported by Fayed et al. (1983), Tewari et al. (1994) and El-Quesni et al. (2002).

Effect of hill-spacing on weed dry weight

The combined data revealed that hill-spacing exerted a significant influence on the weed dry weight. In general, close-sown soybean had a significantly smaller dry weed biomass. The efficiency of the weed control treatments based on the dry weed weight was 73.69%, 77.82% and 79.61% at 45 DAS and 81.88%, 82.82% and 84.57% at 75 DAS with hill-spacings of 5, 10 and 15 cm, respectively (Table 3). Similar results were obtained by Dubey (1998).

Table 2
Combined main effects of weed control treatments and hill-spacing on dry weight of monocot, dicot and total weeds (g/m^2) 45 and 75 DAS

Treatments	45 days after sowing (DAS)			75 days after sowing (DAS)			Total
	Monocot	Dicot	Total	Monocot	Dicot	Total	
Hill-spacing (cm)							
5 cm	33.24	85.74	118.94	51.97	42.34	94.31	213.25
10 cm	41.88	114.72	156.59	63.24	59.77	123.01	279.60
15 cm	65.73	141.39	207.13	85.18	79.13	164.30	371.43
F-test	**	**	*	**	**	**	**
$LSD_{5\%}$	0.53	0.785	0.767	0.628	0.835	1.03	1.33
Weed control treatments							
Trifluralin	5.65	120.49	126.14	16.52	47.88	64.40	190.54
Linuron	27.84	49.70	77.54	56.07	18.31	74.38	151.91
Pendimethalin	2.57	194.08	196.65	1.27	85.18	86.45	283.10
Bentazone	74.02	13.34	87.36	101.30	2.53	103.83	191.19
Hand hoeing (twice)	10.10	2.31	12.34	16.22	1.13	17.35	29.60
Unweeded	161.50	303.78	465.29	209.39	207.45	416.84	882.13
F-test	**	**	**	**	**	**	**
$LSD_{5\%}$	0.64	0.961	1.15	0.658	0.860	1.09	2.05

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively.

Table 3
Combined interaction effects of weed control treatments and hill-spacing (H.S.) on dry weight of monocot, dicot, total weeds and efficiency % 45 and 75 DAS

Treatments		Dry weight of weeds 45 DAS (g)						Dry weight of weeds 75 DAS (g)						Total
H. S.	Weed control	Monocot	Efficiency	Dicot	Efficiency	Total	Efficiency	Monocot	Efficiency	Dicot	Efficiency	Total	Efficiency	
5 cm	Trifluralin	3.74	46.67	96.09	56.86	99.83	70.20	11.06	93.05	36.33	73.62	47.39	84.04	147.21
	Linuron	18.70	83.34	29.93	86.56	48.63	85.48	43.75	72.52	10.78	92.17	54.53	81.63	103.15
	Pendimethalin	1.03	99.08	156.64	29.68	157.66	52.93	1.00	99.57	66.89	51.44	67.89	77.13	225.55
	Bentazone	54.80	51.13	7.90	96.45	62.76	62.76	84.44	46.96	1.16	99.16	85.60	71.17	148.36
	Hand hoeing (twice)	8.86	92.11	1.13	99.49	9.78	97.08	12.38	92.22	1.13	99.18	13.50	95/45	23.28
	Unweeded	112.25		222.75		335.0		159.19		137.76		296.95		631.95
10 cm	Trifluralin	5.79	95.62	127.35	59.37	133.13	70.12	14.44	92.52	50.94	75.02	65.38	83.53	198.50
	Linuron	24.73	81.27	40.55	87.06	65.28	85.35	55.96	71.01	17.94	91.20	73.90	81.39	139.18
	Pendimethalin	3.15	97.61	192.76	38.51	195.91	56.03	1.00	99.48	82.08	59.75	83.08	79.07	278.99
	Bentazone	75.59	42.77	12.19	96.11	87.78	80.29	99.43	48.49	2.62	98.71	102.06	74.29	189.84
	Hand hoeing (twice)	9.93	92.48	2.00	99.36	11.93	97.32	15.55	91.95	1.09	99.46	16.64	95.81	28.56
	Unweeded	132.08		313.46		445.54		193.06		203.94		397.0		842.54
15 cm	Trifluralin	7.44	96.90	138.04	63.20	145.48	76.36	24.06	91.28	56.38	79.91	80.44	85.55	225.91
	Linuron	40.09	83.31	78.63	79.03	118.71	80.71	68.49	75.18	26.21	90.66	94.70	82.98	213.41
	Pendimethalin	3.53	98.53	232.85	37.93	236.38	61.59	1.80	99.35	106.58	62.02	108.38	80.53	344.75
	Bentazone	91.61	61.86	19.93	94.69	111.54	81.87	120.04	56.50	3.79	98.65	123.83	77.75	235.36
	Hand hoeing (twice)	11.53	95.20	3.8	98.98	15.33	97.51	20.74	92.48	1.18	99.58	21.91	96.06	37.24
	Unweeded	240.21		375.13		615.34		275.93		280.64		556.56		1171.90
F-test		**		**		*		**		**		**		**
$LSD_{5\%}$		1.10		1.66		1.99		1.14		1.49		1.89		2.77

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively.

Interaction effect of weed control treatments and hill-spacing on dry weed weight

The interaction between weed control treatments and hill-spacing exerted a significant influence on the dry weed biomass production at both sampling dates. It is clear from these data that hoeing was the best treatment for the control of all weeds, followed by the application of pendimethalin and bentazone to control monocot and dicot weeds, respectively, for plants sown at all hill-spacings at both sampling dates.

Effect of weed control treatments on soybean

Vegetative traits

Plant growth traits, i.e. plant height, number of branches/plant, etc., were affected significantly by the application of weed control treatments when compared with the untreated plants. The results showed that the application of pendimethalin gave the shortest plants (69.2 cm). These results are in agreement with those obtained by Halwanker et al. (1987) and El-Quesni (1993) on soybean. The tallest plants were obtained in the hoeing and trifluralin treatments (79.5 and 78.5 cm, respectively). The differences in plant height may be due to the reduction in stem elongation in these plants (internodal length) (Table 4).

The number of branches/plant was increased by the weed control treatments, especially linuron, which resulted in the highest value (3.79) as compared to the control (2.60). With respect to the location of the first pod, the data showed that hoeing produced the first pod at the lowest node (1.76), followed by the unweeded control (2.81 node), while the other treatments had their first pod at a higher node, especially linuron, pendimethalin and bentazone.

Yield and yield components

The combined data revealed that all the weed control treatments exerted a significant influence on the yield and all the yield components. The data indicated that the highest values of number of pods and weight of pods/plant, seed yield/plant and yield/ha were recorded in the hoeing treatment and after the application of pendimethalin. Hoeing gave the highest weight of pods/plant (25.50 g) over all the weed control treatments. The herbicidal treatments decreased the competition between associated weeds and soybean plants. This resulted in an increase in the metabolites synthesized by soybean leaves; thus, the healthy plants were able to produce a larger number of flowers and consequently a higher numbers of pods/plant as compared to the unweeded treatment. The elimination of weeds by weed control treatments significantly increased the seed yield/ha, resulting in values of 2097.9 kg after hoeing and 1874.2 kg in the pendimethalin treatment as compared to the unweeded treatment (1261.4 kg).

Table 4

Combined main effects of hill-spacing (cm) and weed control treatments on growth traits, yield components and seed composition of soybean grown in the summer seasons of 2000 and 2001

Treatment	Plant height (cm)	No. of branches/ plant	First pod-bearing node	No. of pods/plant	Weight of pods/plant (g)	Weight of seeds/plant (g)	100-seed weight (g)	No. of plants/ha	Seed yield (kg/ha)	Seed oil (%)	Seed protein (%)
Hill-spacing											
5 cm	82.2	2.68	5.13	34.28	13.34	6.43	10.62	300760.6	1941.3	21.38	29.22
10 cm	76.2	3.10	4.13	48.13	20.55	13.27	11.94	127068.2	1695.2	20.00	30.36
15 cm	68.1	4.06	3.08	72.77	29.67	18.74	13.69	66211.6	1242.1	18.45	31.01
F-test	**	**	**	**	**	**	**	**	**	**	**
$LSD_{5\%}$	1.06	0.125	0.106	1.97	0.38	0.394	0.134	0.90	12.80	0.195	0.32
Weed control treatments											
Trifluralin	78.5	3.09	3.61	47.21	20.10	12.90	11.17	168527.8	1676.2	20.37	29.94
Linuron	76.0	3.79	5.25	51.22	18.19	12.09	15.91	163553.6	1472.9	21.16	32.06
Pendimethalin	69.2	3.69	5.31	65.41	24.83	13.54	15.39	173668.6	1874.2	20.00	30.04
Bentazone	72.6	3.12	5.94	49.44	19.44	12.12	11.24	155628.2	1374.4	19.9	29.72
Hand hoeing (twice)	79.5	3.38	1.76	59.24	25.50	14.15	13.01	183902.6	2097.9	20.69	30.99
Unweeded	77.5	2.60	2.81	37.83	19.07	12.06	9.79	142800.0	1261.4	17.54	28.43
F-test	**	**	**	**	**	**	**	**	**	**	**
$LSD_{5\%}$	1.66	0.127	0.151	1.39	0.71	0.354	0.249	1.23	23.13	0.301	0.26

** Significant at the 0.01 level of probability

This superiority might be mainly due to the greater suppression of total weeds and the higher seed yield per unit area and its related components. Weeds compete directly with soybean plants for light, water and minerals and consequently reduce the amount of metabolites synthesized by the plant. This in turn causes a depression in the growth of soybean plants, as indicated by the plant height and weight. These results are in harmony with those obtained by El-Deeb et al. (1987), Gaweesh (1987), Hassanein et al. (1988) and Shaban et al. (1991), who indicated that the best treatments were pendimethalin at 1.785 kg ha^{-1} combined with oxyflourfen at 0.856 kg or with linuron at 0.880 kg a.i./ha^{-1}.

Chemical composition

The data revealed that the oil and protein percentages in soybean seeds were significantly affected by the weed control treatments. The highest values of protein (32.06%) and oil (21.16%) were obtained when linuron was applied (Table 4). Hoeing and pendimethalin treatment produced similarly high values for protein (30.99 and 30.04%), while trifluralin and bentazone produced the lowest values of protein (29.94 and 29.72%), though this was higher than the unweeded control. The stimulation effect of herbicides on the nitrogen metabolism could be related to the activation of the enzymes involved in nitrate reduction and/or carbohydrate utilization for amino acid and protein synthesis (Pulver and Ries, 1973).

The effect of such treatments on the protein and oil content in soybean seeds may be due to their effects on the growth and development of plants from planting to harvest. The compounds may exert an effect on vegetative growth and consequently increase the synthesis of metabolites required for protein and oil biosynthesis.

Effect of hill-spacing

Vegetative growth traits

The combined data revealed that hill-spacing exerted a significant influence on plant height, number of branches/plant and location of the first pod. In general, increasing the hill-spacing to 15 cm tended to produce shorter plants (68.1 cm) and increase the number of branches/plant (4.06) as compared to closer spacing. In closer spacing, the competition between plants for light and other environmental factors was high and forced the plants to grow higher as the result of increased plant hormones, while under wider spacing, competition between the plants was lower and the amount of hormones decreased as the result of the increased light intensity under these conditions. The lateral buds were more active and tended to form a larger number of branches/plant. As regards the location of the first pod, at lower plant density the first pod was produced at a lower node as a result of decreasing internodal length as compared to plants grown at higher density. Parvez et al. (1989) reported that an increase in plant density decreased the number of branches/plant and the height of the first pod in soybean.

Yield and yield components

The combined data showed that hill-spacing exerted a significant influence on the yield and yield components. Increasing the hill-spacing to 15 cm increased the number and weight of pods/plant (72.77, 29.67 g), the seed index (13.69 g) and the seed yield/plant (18.74 g) as compared to closer spacing (5 cm) (Table 4). This means that increasing the distance between the hills increased the number of branches/plant (4.06) and resulted in the first pod being produced on a lower internode (3.08 node), thus increasing the number of pods and the seed setting in the pods. Seed size was also greater in such plants. The increase in plant density per unit area increased competition between the plants and consequently reduced the photosynthetic activity of the leaves, forcing the plants to produce fewer pods with lower seed yield. However, the seed yield/ha was higher at close spacing (5 cm) than at wider spacing (15 cm). This may be due to the increase in the number of plants at harvest (300760.6 per ha), which consequently produced the highest yield (1941.36 kg/ha). Duncan (1986) stated that a grrater seed yield may be obtained in soybean due to greater light interception and dry matter production before seed initiation. Thus, factors that control plant size, such as narrow-row spacing, tend to increase the yield. Parvez et al. (1989) also found that an increase in the plant density of soybean resulted in an increase in the total seed yield.

Chemical composition

Hill-spacing exerted a significant influence on the protein and oil content of soybean seeds. In the case of protein, the data showed that wider spacing enhanced the formation of protein in the seeds, while the reverse was true for the oil percentage. This means that under wider spacing the plants were able to form more metabolites to synthese more protein in the seeds, and the activity of protein synthesis was higher than at closer spacing.

Interaction of weed control treatments and hill-spacing

Vegetative growth traits

The effect of the interaction between weed control treatments and hill-spacing was significant for the growth traits studied. The tallest plants (87.2 cm) were obtained with hoeing when the plants were sown at 5 cm between hills, while the highest value of branches/plant (4.43) was obtained with linuron when the plants were sown at 15 cm. As for the location of the first pod, the lowest value (1.5) was obtained with hoeing when the plants were sown at 15 cm between hills.

Table 5

Combined interaction effects of hill-spacing (H.S., cm) and weed control treatments on growth traits, yield components and seed composition of soybean grown in the summer seasons of 2000 and 2001

Treatment		Plant height (cm)	No. of branches/ plant	First pod-bearing node	No. of pods/plant	Weight of pods/plant (g)	Weight of seeds/plant (g)	100-seed weight (g)	No. of plants/ha	Seed yield (kg/ha)	Seed oil (%)	Seed protein (%)
H.S.	Weed control											
5	Trifluralin	84.4	2.587	4.448	33.44	15.39	6.37	10.03	306924.8	1950.6	21.87	29.15
	Linuron	82.8	3.184	6.697	38.61	13.44	5.48	13.82	299808.6	1644.5	22.93	31.20
	Pendimethalin	76.1	3.184	6.297	52.53	14.78	7.43	11.09	315135.8	2349.0	21.37	29.05
	Bentazone	77.9	2.313	7.746	21.45	10.20	5.45	9.69	289051.0	1569.1	21.10	28.30
	Hand hoeing (twice)	87.2	2.811	2.045	36.63	16.16	8.52	11.77	327630.8	2728.6	21.70	30.48
	Unweeded	84.5	1.990	3.573	23.00	10.07	5.30	7.31	266012.6	1405.1	19.31	27.12
10	Trifluralin	82.3	2.935	3.573	44.33	17.94	13.69	11.11	130757.2	1799.0	20.43	30.28
	Linuron	76.7	3.781	5.447	46.20	17.67	12.03	15.45	127092.0	1533.9	20.00	32.04
	Pendimethalin	68.7	3.582	5.247	74.37	26.26	14.17	11.74	135517.2	1928.0	20.36	30.00
	Bentazone	72.6	2.811	6.047	40.06	17.10	12.43	10.31	116929.4	1438.4	20.6	29.72
	Hand hoeing (twice)	79.6	2.985	1.749	46.79	24.45	14.56	12.71	147345.8	2140.3	20.6	31.12
	Unweeded	77.4	2.488	2.699	37.06	19.87	12.71	10.34	104720.0	1331.3	17.98	29.03
15	Trifluralin	68.8	3.756	2.805	63.88	26.97	18.63	12.38	6783.8	1279.2	18.81	30.38
	Linuron	68.4	4.428	3.598	68.85	23.46	18.77	16.89	63736.4	1240.4	20.04	32.96
	Pendimethalin	63.0	4.303	4.398	69.34	33.46	19.01	12.90	70352.8	1344.2	18.26	31.08
	Bentazone	67.3	4.229	4.023	86.80	31.01	18.48	13.71	60904.2	1115.7	18.34	31.16
	Hand hoeing (twice)	70.6	4.328	1.499	94.29	35.89	19.36	14.56	76707.4	1424.9	19.92	31.36
	Unweeded	70.6	3.308	2.149	53.44	27.25	18.17	11.71	57691.2	1048.1	15.31	29.15
F-test		**	**	**	**	**	**	**	**	**	**	
$LSD_{5\%}$		2.87	0.220	2.262	2.41	1.24	0.615	0.430	2.14	40.12	0.523	

** Significant at the 0.01 level of probability.

Yield of soybean

The combined data revealed that the interaction between hill-spacing and herbicide treatments exerted a significant influence on the yield and yield components. In general, the highest seed yield (2728.6 kg/ha) was obtained with hoeing when the plants were sown at 5 cm between hills (Table 5).

Chemical composition

The interaction exerted a significant influence on the protein and oil percentages in soybean seeds. In general, the highest protein content was obtained after the application of linuron when the soybean plants were sown at 10 or 15 cm (32.04 and 32.96%) (Table 5), while for oil, the highest value (22.93%) was obtained by applying linuron when the plants were sown at 5 cm. This means that more protein was obtained at lower density and more oil at higher density when linuron was applied.

Conclusions

The results of this work suggested that the highest yield of soybean could be obtained by dense planting and hand hoeing or by applying pendimethalin herbicidal treatment.

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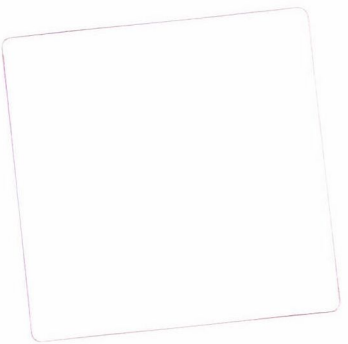

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EFFECT OF SPAD TECHNIQUES AND PLANTING DENSITY ON 'Y' LEAF NITROGEN CONCENTRATION IN TRANSPLANTED RICE

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Field experiments were conducted in June–September 1998 and 1999 with rice variety ASD18 at the wetland farm of Tamil Nadu Agricultural University, in Coimbatore, India to examine variations in 'Y' leaf (youngest fully expanded leaf) N concentration as influenced by different planting densities and N management strategies in a split plot design. The main plot consisted of three plant populations (33, 66 and 100 hills m^{-2}) and the sub-plots treatments of five N management approaches. The results revealed that the nitrogen concentration progressively declined with growth, the decline being steep up to 35 days after transplanting, wereafter the values became almost linear up to the flowering stage in all the treatments. The mean 'Y' leaf N was found to be significantly higher at 33 hills m^{-2} (45.1 g kg^{-1}), while the other two densities were on par (42.9 g kg^{-1}). When N application was based on chlorophyll meter (SPAD) values the leaf N concentration was maintained at a level of 39.2 to 51.9 g kg^{-1} to produce maximum grain yield. A significant correlation was observed between the chlorophyll meter values and 'Y' leaf N concentrations at various days after transplanting (r values ranged from 0.57* to 0.83**), while the correlation was highly significant during the major physiological growth stages. Though the 'Y' leaf content was significantly higher in the treatment involving *Sesbania rostrata* green manuring + 150 kg N applied in splits, the grain yield produced was on par in all the N applied treatments. A highly significant correlation was observed between the grain yield and both 'Y' leaf N content and SPAD values during various growth periods.

Key words: SPAD value, 'Y' leaf N, transplanted rice, planting density

Introduction

Nitrogen topdressing to rice based on chlorophyll meter (SPAD-502) readings is one of the recently developed N management practices (IRRI, 1995). This meter was developed by the Soil-Plant Analysis Development (SPAD) of the Minolta Company, Japan, and is hence also called a SPAD meter and the method as the SPAD method or SPAD technique. It is a non-destructive method involving the measurement of the greenness of the youngest fully expanded leaf ('Y' leaf). In this approach, N is applied when the chlorophyll meter values fall below the predetermined threshold value. Green leaf colour was calibrated very precisely to the leaf N content (Tanno, 1988). There is a strong linear relationship between SPAD values and weight-based leaf N concentration, but this varies with the crop growth stage and/or the variety (Takebe and Yoneyama, 1989; Turner and Jund, 1991). A relationship between 'Y' leaf N concentration and the grain yield of rice cultivars grown in the southern USA was reported by Brandon et al. (1982) and Brandon and Wells (1986). Hence this study was carried out to examine the influence of different planting densities and various N management approaches on the 'Y' leaf N concentration as measured with a chlorophyll meter (SPAD meter) and a laboratory method, and its relationship with grain yield production.

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Materials and methods

Two field experiments were conducted in June–September 1998 and 1999 with rice variety ASD18 at the wetland farm of Tamil Nadu Agricultural University in Coimbatore, India. Coimbatore is situated in the Northwestern agroclimatic zone of Tamil Nadu at 11°N latitude and 77°E longitude and at an altitude of 426.7 m above mean sea level. The sowing and transplanting dates of the crop were the same in both the years. The trials were located in two different fields of the same farm in 1998 and 1999, and the characteristics of the soils of the two fields are given in Table 1, as analysed by the procedure given by Jackson (1973).

A split-plot design with three replications was used. Three planting densities, 33 (30 × 10 cm); 66 (15 × 10 cm) and 100 (10 × 10 cm) hills m^{-2}, served as the main plots and five N regimes, Control (Minus-N), *Sesbania rostrata* green manure (SGM) at 6.25 t ha^{-1} + 150 kg N ha^{-1} (SGM+N), SPAD-guided N topdressing (SPAD), basal N at 25 kg ha^{-1} + SPAD-N (Basal+SPAD) and SGM at 6.25 t ha^{-1} + SPAD-N (SGM+SPAD) as the sub-plots. Fertilizer N was applied in four splits, i.e. 25% at 7 days after transplanting, 25% at the active tillering stage (21 days after transplanting), 25% at panicle initiation (42 days after transplanting) and the remaining 25% at 10 days after panicle initiation. In all the SPAD-N treatments, SPAD readings were taken at 7-day intervals starting from 14 days after transplanting until first flowering, using the youngest fully expanded leaf ('Y' leaf) from ten randomly selected plants from each plot. After each SPAD observation, the leaves on which SPAD measurements were made ('Y' leaf) were collected immediately after the measurements and pooled for each plot to estimate the N concentration in the laboratory.

In the case of SPAD management, N application as topdressing commences only from 14 DAT if the observed chlorophyll meter value falls below the set threshold value. In the existing recommended N application strategies, basal N application amounting to as much as 25–50% of the total N to be applied is recommended besides 6.25 t ha^{-1} of green manure. The SPAD threshold value was set at 37.

The crop was harvested at physiological maturity and the grain yield from the individual plots was recorded separately, the yield being converted to 14% moisture content. The concentration of N in the grain sample was analysed by the Kjeldahl digestion method, using the required quantity of a 1000:100:1 digestion mixture of K_2SO_4 : $CuSO_4$: Se and concentrated H_2SO_4, as described by Jones and Case (1990), using a Kjelplus instrument for distillation. The titration was done manually using 0.02 N H_2SO_4. The uptake was calculated from the N content and biomass calculated. The data obtained for both the years was pooled and statistically analysed using the Computer software IRRISTAT (IRRI, 1993). The correlation and regression analysis were done using the procedure given by Gomez and Gomez (1984).

Table 1
Characteristics of the soils (pre-puddling) of the experimental sites

Soil characteristics	1998	1999
Clay (%)	46.2	41.8
Silt (%)	8.7	12.1
Sand (%)	45.1	46.1
pH	7.9	8.1
Electrical conductivity (dSm^{-1})	0.4	0.5
Cation exchange capacity (cmol kg^{-1})	17.3	21.2
$KMnO_4$-extractable N (kg ha^{-1})	214	163
Olsen-P (kg ha^{-1})	16	13
NH_4OAc-extractabe K (kg ha^{-1})	400	425
Total N (g kg^{-1})	5.22	3.86

Results and discussion

Chlorophyll meter values

In spite of the fact that the N was supplied basally (Table 2) in the form of SGM and urea, the chlorophyll meter values were unable to reach the threshold value at 14 days after transplanting. This showed that either the N demand of the crop could not be met from the soil and applied sources together, or the plants were not able to acquire N to the extent of increasing the leaf N concentration. The time course of chlorophyll meter values showed that the values were below the threshold level at 14 days after transplanting under all the N regimes (Table 3).

The chlorophyll meter values at different growth stages were significantly influenced by the differences in planting density. The significantly highest and lowest values were recorded for 33 and 100 hills m^{-2}, respectively. In spite of the lower rate of N application (Table 2), the chlorophyll meter values were maintained above the threshold level from 21 to 56 days after transplanting in the 33 hills m^{-2} treatment. The mean chlorophyll meter values at 35 and 42 days after transplanting were below the threshold value of 37 in the case of 100 hills m^{-2}. This can be attributed to the dilution of the N concentration due to higher biomass production at 100 hills m^{-2} than at 33 hills m^{-2}. The application of N had a significant effect on the chlorophyll meter values and the maximum value was observed 28 days after transplanting under all the N regimes except SGM+N, which showed a maximum value 49 days after transplanting. This can be attributed to the effect of the N (50 kg N ha^{-1}) applied 42 days after transplanting under all densities (Table 2). The time course of chlorophyll meter values under the three SPAD-guided N regimes were similar, indicating the effect of need-based N application using a chlorophyll meter.

'Y' leaf N concentration

The nitrogen concentration in the 'Y' leaf (on which SPAD measurements were made) at different intervals ranged from 32.0 to 60.4 g kg^{-1} in various N regimes (Table 4). The average 'Y' leaf N varied significantly between planting densities, N regimes and growth stages. The nitrogen concentration progressively declined with growth, the decline being steep up to 35 days after transplanting, whereafter the values became almost linear (Fig. 1) up to the flowering stage.

Table 2
Cumulative total N applied (kg ha^{-1}) in the different N management treatments and planting densities

Planting density (hills m^{-2})	Cumulative total N applied (kg ha^{-1})							
	SGM*+N		SPAD-N		Basal+SPAD		SGM*+SPAD	
	1998	1999	1998	1999	1998	1999	1998	1999
33	193	201	90	105	85	130	73	126
66	193	201	105	135	130	160	118	156
100	193	201	105	135	100	145	148	171

*The quantity of total N supplied through 6.25 t ha^{-1} (fresh weight) of green manure (*Sesbania rostrata*) was 43 kg in 1998 and 51 kg in 1999.

Table 3
SPAD values at various days after transplanting as influenced by different N regimes and planting densities

Planting density (hills m^{-2})	14DAT		21DAT		28DAT		35DAT		42DAT		49DAT		56DAT	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
Minus-N														
33	30.9	33.0	34.5	34.8	35.6	37.4	35.4	36.0	38.5	33.7	37.2	34.4	36.3	34.2
66	30.7	34.0	34.8	33.8	34.1	33.3	32.7	34.1	34.1	32.4	34.9	33.3	35.6	30.8
100	31.3	32.6	33.8	32.5	34.2	31.2	32.7	32.6	32.5	30.8	33.4	33.2	35.2	30.9
Mean	30.96	33.2	34.4	33.7	34.6	33.9	33.6	34.2	35.0	32.3	35.2	33.6	35.7	31.9
SGM+N														
33	30.58	32.1	35.6	36.8	41.3	41.1	40.9	40.4	40.2	35.9	41.9	42.2	44.0	42.7
66	31.7	34.4	37.6	37.7	39.8	39.8	39.0	39.6	37.0	35.2	42.5	39.6	42.4	40.4
100	34.1	34.3	36.2	36.4	40.6	39.3	37.4	37.4	35.1	33.1	40.4	37.5	40.4	38.5
Mean	32.1	33.6	36.5	36.9	40.6	40.1	39.1	39.1	37.4	34.7	41.6	39.8	42.2	40.5
SPAD														
33	30.3	33.3	36.3	39.9	40.6	37.8	38.1	37.0	37.38	42.2	36.7	39.1	39.7	34.0
66	30.1	33.0	35.4	37.6	39.6	36.2	36.4	40.6	40.4	37.8	38.6	35.4	39.2	36.2
100	31.9	33.2	36.0	36.0	40.1	38.4	36.5	35.6	40.1	38.4	39.4	37.3	37.6	35.0
Mean	30.8	33.2	36.1	37.8	40.1	37.4	37.0	37.7	39.4	39.5	38.2	37.3	38.8	35.1
Basal+SPAD														
33	33.9	35.5	36.9	39.3	40.6	38.2	38.1	36.6	38.1	39.1	37.8	38.8	38.0	34.1
66	31.3	36.8	37.3	38.4	39.4	34.7	36.5	38.6	40.6	37.3	40.2	35.9	39.5	36.5
100	32.4	36.2	37.7	38.8	37.3	33.9	33.3	36.9	38.3	36.2	40.5	37.7	39.3	37.8
Mean	32.5	36.2	37.3	38.8	39.1	35.6	36.0	37.4	39.0	37.5	39.5	37.4	38.9	36.1
SGM+SPAD														
33	30.85	33.7	38.5	39.5	38.7	37.2	39.3	40.8	37.8	37.9	39.2	38.3	37.3	37.5
66	31.5	33.3	38.0	39.4	36.8	36.6	36.0	40.0	40.7	37.3	39.4	35.5	39.7	37.7
100	31.9	34.7	36.5	38.4	39.4	36.0	35.1	37.7	40.3	36.4	39.2	37.6	38.1	37.6
Mean	31.3	33.9	37.7	39.1	38.3	36.6	36.8	39.5	39.3	37.2	39.3	37.2	38.4	37.6
Stage Mean	31.5	34.0	36.3	37.2	38.5	36.7	36.4	37.6	38.1	36.2	38.7	37.0	38.8	36.3

Pooled analysis

Source	SED	$LSD_{5\%}$	Source	SED	$LSD_{5\%}$
Growth stage (S)	0.25	0.49	S*N	0.49	0.96
Planting density (D)	0.16	0.32	D*N	0.32	0.63
N regimes (N)	0.18	0.36	S*D*N	0.84	1.66
S*D	0.42	0.85			

Table 4

'Y' leaf N (g kg^{-1}) at various days after transplanting as influenced by different N regimes and planting densities

Planting density (hills m^{-2})	14DAT		21DAT		28DAT		35DAT		42DAT		49DAT		56DAT	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
Minus-N														
33	46.7	50.3	46.0	44.5	43.3	37.0	39.7	36.3	42.1	32.5	39.5	33.0	30.3	37.3
66	51.6	49.4	45.6	38.5	36.9	36.7	35.3	34.6	34.4	31.2	38.8	33.1	33.8	30.9
100	51.9	44.3	46.8	40.5	41.6	33.1	38.5	33.3	36.2	27.8	33.7	30.1	36.3	30.1
Mean	50.1	48.0	46.1	41.2	40.6	35.6	37.8	34.7	37.6	30.5	37.3	32.0	33.4	32.8
SGM+N														
33	51.8	51.7	50.5	51.8	57.5	52.8	47.9	40.8	43.5	38.9	48.5	45.9	45.0	44.8
66	49.1	55.5	49.2	47.4	55.2	50.3	43.4	40.4	37.5	35.3	50.3	44.3	45.0	45.7
100	55.1	55.1	49.9	54.0	53.9	48.9	41.0	37.2	36.1	34.2	46.6	40.8	40.2	43.9
Mean	52.0	54.1	49.8	51.1	55.6	50.7	44.1	39.5	39.0	36.1	48.5	43.7	43.4	44.8
SPAD														
33	46.8	51.1	53.2	52.8	54.0	46.4	41.8	44.6	41.4	51.1	38.9	37.1	42.4	32.5
66	47.3	50.0	50.3	47.1	53.3	40.2	39.3	43.8	44.6	39.4	43.6	34.7	41.9	37.4
100	52.3	47.1	52.2	43.8	47.8	42.0	38.4	36.5	46.6	46.0	41.5	34.0	39.1	34.7
Mean	48.8	49.4	51.9	47.9	51.7	42.9	39.8	41.6	44.2	45.5	41.3	35.3	41.1	34.9
Basal+SPAD														
33	51.8	68.9	50.2	57.8	50.6	42.2	44.0	40.9	40.2	41.0	40.8	36.0	39.2	32.8
66	49.4	55.3	49.7	48.5	48.3	40.9	39.4	40.5	46.4	37.7	41.8	33.6	39.2	39.6
100	51.4	61.4	54.9	47.3	44.9	36.6	38.1	44.3	43.5	36.3	42.6	40.2	42.9	35.3
Mean	50.9	61.9	51.6	51.2	47.9	39.9	40.5	41.9	43.3	38.3	41.7	36.6	40.4	35.9
SGM+SPAD														
33	51.0	55.5	55.8	58.9	49.6	46.5	45.4	51.1	40.3	41.1	44.0	39.7	39.1	41.5
66	50.6	49.5	52.9	50.1	44.9	41.0	34.1	46.6	41.6	36.4	41.6	38.7	41.8	37.1
100	53.1	53.3	50.8	51.7	51.4	38.5	37.5	45.6	44.6	32.1	44.2	35.0	43.7	38.2
Mean	51.6	52.8	53.2	53.6	48.6	42.0	38.9	47.8	42.2	36.5	43.2	37.8	41.5	38.9
Stage Mean	50.3	55.5	50.5	48.9	48.8	42.2	40.2	41.0	41.2	37.4	42.3	37.0	39.9	37.4

Pooled analysis

Source	SED	$LSD_{5\%}$	Source	SED	$LSD_{5\%}$
Growth stage (S)	0.57	1.134	S*N	1.01	1.99
Planting density (D)	0.37	0.74	D*N	0.67	NS
N regimes (N)	0.80	0.76	S*D*N	1.76	3.46
S*D	0.98	1.97			

The main effects of planting densities and N regimes were highly significant, while the interaction between planting densities and N regimes was not significant. This showed that there was a tendency for the crop to maintain 'Y' leaf N at a certain level irrespective of the plant population. Similarly, Sinclair and Horie (1989) found that for each rate of N supply to the leaves, an optimum leaf N content existed to maximize crop biomass accumulation. The mean 'Y' leaf N was found to be significantly higher at 33 hills m^{-2} (45.1 g kg^{-1}), while the other two densities were on par (42.9 g kg^{-1}). This can be attributed to the dilution of the N concentration due to higher biomass production at 100 hills m^{-2} than at 33 hills m^{-2} (Fig. 2), as observed for the SPAD values. The variation in root/shoot ratio with resource availability may also directly affect the leaf N concentration (Hilbert, 1990).

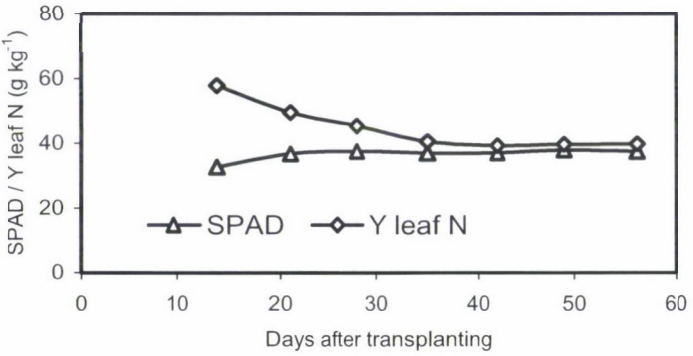

Fig. 1. Time course of SPAD values and 'Y' leaf N concentration in transplanted rice variety ASD18

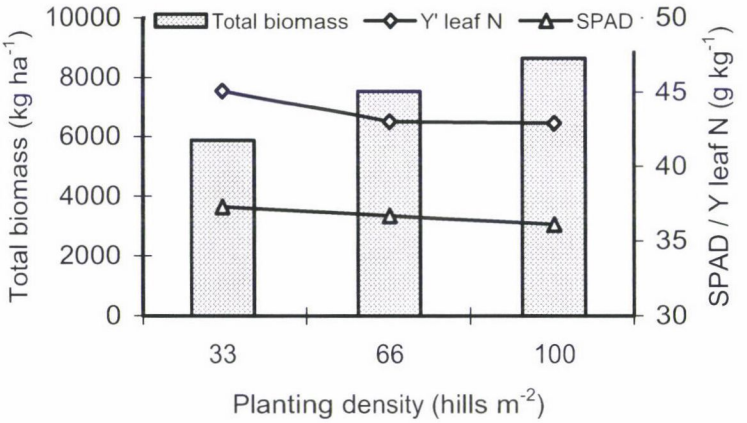

Fig. 2. Influence of planting density on 'Y' leaf N content and total biomass production

The SGM+N treatment gave the highest 'Y' leaf N content (47.5 and 45.7 g kg^{-1} in 1998 and 1999, respectively), while the effects of the other N regimes were on par. This higher value in the SGM+N treatment could be ascribed to the highest rate of N application during different growth stages. The on-par values for all planting densities in the three SPAD-guided N regimes confirmed the advantage of need-based N application, which maintained the leaf N concentration at a certain level to produce maximum grain yield. A similar report was given by Hilbert (1990), who found that the regulation of leaf N concentration was an important means by which plants could adjust their physiological status to the prevailing environment (Figs 3, 4).

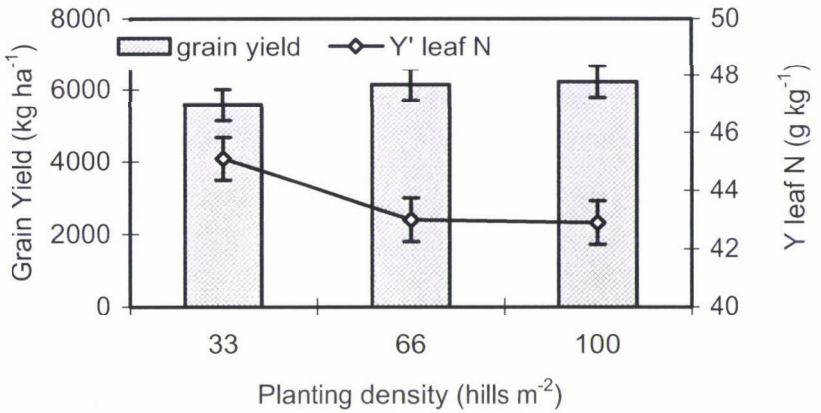

Fig. 3. Influence of planting density on 'Y' leaf N content and grain yield in transplanted rice (Error bars indicate the LSD values at the 5% level of significance)

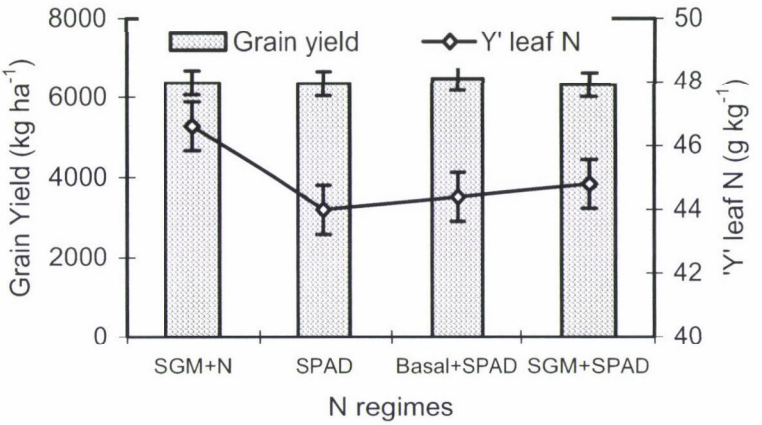

Fig. 4. Influence of different N application strategies on 'Y' leaf N content and grain yield in transplanted rice (Error bars indicate the LSD values at the 5% level of significance)

Relationship between SPAD and 'Y' leaf N

A highly significant correlation was found to exist between the chlorophyll meter values and 'Y' leaf N concentrations at various days after transplanting (Table 5). Therefore, the regression equation obtained (Table 5) can be used to predict the 'Y' leaf N concentration for a given SPAD value at a particular growth stage in the transplanted rice variety ASD18. Further, the SPAD technique may also be used to diagnose the N status of rice plants, providing a useful tool for managing research experiments and varietal trials, where an adequate N supply must be maintained in diverse environments and on different soils.

Though the quantity, source and time of N application (Table 5) varied for the three SPAD-guided N regimes at different planting densities, the mean 'Y' leaf N contents recorded were on par (Table 2), indicating the regulation of 'Y' leaf N under SPAD-based N management practices.

Table 5
Relationship between SPAD and 'Y' leaf N, SPAD and grain yield, and 'Y' leaf N and grain yield during different growth periods and for the pooled data using all the growth periods

Growth periods	SPAD vs. 'Y' leaf N	SPAD vs. grain yield	'Y' leaf N vs. grain yield
14 DAT			
Correlation coefficient (r)	0.57*	0.39	0.31
Regression coefficient (R^2)	0.32	0.12	0.08
Regression equation	y = 1.588 x	y = 182.62 x	y = 114.93 x
21 DAT (Tillering stage)			
Correlation coefficient (r)	0.70**	0.72**	0.55*
Regression coefficient (R^2)	0.48	0.35	0.30
Regression equation	y = 1.346 x	y = 162.8 x	y = 120.19 x
28 DAT			
Correlation coefficient (r)	0.81**	0.55*	0.49*
Regression coefficient (R^2)	0.57	0.28	0.14
Regression equation	y = 1.212 x	y = 159.1 x	y = 131.17 x
35 DAT			
Correlation coefficient (r)	0.75**	0.52*	0.42
Regression coefficient (R^2)	0.57	0.26	0.16
Regression equation	y = 0.566 x	y = 161.59 x	y = 146.81 x
42 DAT (Panicle initiation)			
Correlation coefficient (r)	0.82**	0.64**	0.54*
Regression coefficient (R^2)	0.64	0.36	0.27
Regression equation	y = 1.059 x	y = 161.18 x	y =151.66 x
49 DAT			
Correlation coefficient (r)	0.83**	0.71**	0.54*
Regression coefficient (R^2)	0.64	0.41	0.28
Regression equation	y = 1.050 x	y = 158.2 x	y = 149.88 x
56 DAT (Flowering stage)			
Correlation coefficient (r)	0.78**	0.68**	0.73**
Regression coefficient (R^2)	0.59	0.42	0.53
Regression equation	y = 13.033 x	y = 159.61 x	y = 154.6 x
TOTAL			
Correlation coefficient (r)	0.93**	0.78**	0.72**
Regression coefficient (R^2)	0.79	0.38	0.45
Regression equation	y = 1.191 x	y = 163.43 x	y = 137.53 x

DAT: Days after transplanting; * and **: significant at the 5 and 1% level respectively.

Though the 'Y' leaf content was significantly higher in the SGM+N regime, the grain yield produced was on par in all the N regimes. This could be due to the increased photosynthetic rate and higher partitioning of N to panicle production caused by the maintenance of an adequate level of 'Y' leaf N concentration during different growth stages. The importance of the N concentration in rice leaves for photosynthesis has been well documented (Sinclair and Horie, 1989; Greenwood et al., 1991), as has its significance for grain yield (Thiyagarajan et al., 1994). Highly significant correlations were observed between the grain yield and both the 'Y' leaf N content and the SPAD values during different growth periods (Table 3), with r values ranging from 0.39 to 0.72 and 0.31 to 0.73, respectively. This showed the favourable influence of SPAD-based N application in enhancing the grain yield and confirmed the above findings. The regression equations obtained can be used to predict the grain yield expected for various SPAD values or 'Y' leaf N concentrations.

It can be concluded from the above study that an increase in planting density decreases the 'Y' leaf N concentration. This result was significant at lower planting densities. The application of N to transplanted rice based on the SPAD technique ensured the N supply as and when there was a demand by the crop and increased the grain yield by maintaining adequate 'Y' leaf N concentrations during different physiological growth stages irrespective of the planting density. Further, the significant correlation between SPAD values and 'Y' leaf N content, and between these parameters and the grain yield demonstrates the advantage of need-based N application using a chlorophyll meter. Among the different SPAD techniques, SPAD-based N application starting from 14 days after transplanting onwards is the best practice, as it reduces the amount of fertilizer N applied without reducing the grain yield.

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Short communication

EFFECT OF STUBBLE MANAGEMENT WITH BIOLOGICAL INOCULANTS ON THE GROWTH AND YIELD OF RICE (*Oryza sativa* L.) IN RICE-BASED CROPPING SYSTEMS

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A field experiment was conducted at Tamil Nadu Agricultural University from July 2001 to July 2002 to study the effect of different stubble management practices using biological inoculants on the growth and yield of rice in rice-based cropping systems. Inoculation with *Trichoderma viride* during stubble incorporation followed by the application of 120 kg N ha^{-1} in 4 splits produced significantly taller plants, higher LAI and dry matter, a larger number of productive tillers, longer panicles with more filled grains and higher grain yield. However, it was on par with the stubble management practice involving *Trichoderma viride* followed by the application of 90 kg N ha^{-1} in 4 splits.

Key words: rice, stubble management, biological inoculants, N levels and splits, yield

Introduction

Organic recycling plays a major role in supplementing essential nutrients for maintaining soil fertility. Rice straw and stubbles are potential sources of nutrients in rice-based cropping systems, since they are locally available *in situ* in large quantities. Rice straw contains 42% C, 40% cellulose, 22% hemicellulose, 0.6% N, 0.1% P and 1.3% K (Mishra et al., 2001). Their direct incorporation into the soil leads to the poor establishment of the succeeding crop, which ultimately lowers the yield of the crop, due to the initial immobilization of nitrogen and to the toxic substances released during intermediary decomposition (Udayasoorian et al., 1997). The use of cellulose-degrading fungi (Udayasoorian et al., 1997) and *Pleurotus sajor-caju* for lignin degradation (Hitoichi, 1997) could be beneficial, as they are effective in decomposing rice straw and could speed up nutrient availability by restoring the physical and chemical properties of the soil, while also producing some growth regulators which are beneficial for the rice crop, thereby improving the yield of rice. It was against this background that the present investigation was undertaken to study the effect of stubble management practices with biological inoculants, namely *Trichoderma viride* and *Pleurotus sajor-caju*, and different N levels and splits on the growth and yield of rice.

0238–0161/$ 20.00

Materials and methods

A field experiment was conducted on the wetland farm of Tamil Nadu Agricultural University, Coimbatore between July 2001 and July 2002. In July 2001, prior to the monsoon rains a bulk crop of hybrid rice (CORH-2) was sown as the first crop in the cropping system and was harvested leaving a uniform stubble 20 cm in height in the field. Stubble samples were collected from the field and oven dried for the estimation of the biomass added. A total of 6.25 t ha^{-1} stubble was incorporated, making up the quantity with straw from elsewhere, one month prior to planting the main rice crop (ADT-36), on which the treatments were imposed. During the incorporation of the stubble, the biological inoculants, *Trichoderma viride* and *Pleurotus sajor-caju* were sprinkled at a rate of 1 kg t^{-1} of stubble biomass to promote the faster decomposition of the stubble.

The soil was clay (Typic Haplustalf) having pH 8.0, electrical conductivity 0.40 dsm^{-1}, organic carbon 0.59% and available N, P and K of 248, 15 and 550 kg ha^{-1}, respectively. The experiment was laid out in a randomized block design with three replications. There were ten treatments: T_1, 120 kg N ha^{-1} in 3 splits + *Trichoderma viride*; T_2, 120 kg N ha^{-1} in 3 splits + *Pleurotus sajor-caju*; T_3, 90 kg N ha^{-1} in 3 splits + *Trichoderma viride*; T_4, 90 kg N ha^{-1} in 3 splits + *Pleurotus sajor-caju*; T_5, 120 kg N ha^{-1} in 4 splits + *Trichoderma viride*; T_6, 120 kg N ha^{-1} in 4 splits + *Pleurotus sajor-caju*; T_7, 90 kg N ha^{-1} in 4 splits + *Trichoderma viride*; T_8, 90 kg N ha^{-1} in 4 splits + *Pleurotus sajor-caju*; T_9, Recommended N alone (120 kg N ha^{-1}) in 3 splits without any biological inoculants and T_{10}, control (only stubble incorporation at 6.25 t ha^{-1} without biological inoculants or fertilizer application). Nitrogen in the form of urea was applied in 3 splits (50% as basal, 25% each at 30 and 45 days after transplanting) or 4 splits (25% each at basal, 15, 30 and 45 days after transplanting). The recommended rates of P and K (38 kg ha^{-1}) were applied as base fertiliser in the form of single superphosphate and muriate of potash, respectively.

Results and discussion

Growth and developmental characters

Stubble management practices using biological inoculants had a marked effect on the growth and developmental characters of rice (Table 1). Stubble management with *Trichoderma viride* and the application of 120 kg N ha^{-1} in 4 splits (T_5) gave maximum plant height, and an increase in the number of tillers $hill^{-1}$, leaf area index (LAI) and dry matter production (DMP). However, it was on par with stubble management with *Trichoderma viride* and the application of 90 kg N ha^{-1} in 4 splits (T_7). This may be due to the beneficial effect of the cellulolytic fungus, *Trichoderma viride,* favouring the rapid decomposition of stubble and straw, and thereby promoting a steady, continuous supply of nutrients in available form for a longer period of time. This in turn increased the nutrient uptake and stimulated the development of an elaborate root system, as a consequence of which the vigour of the plants was well maintained throughout crop growth. Bacon and Lewin (1990) also reported similar results.

The increase in LAI may be due to the greater N supply provided by combining organic sources (decomposition of straw) and inorganic fertilizer N, which led to the production of more leaves of larger size, thus resulting in higher LAI and DMP. This is in agreement with the findings of Sharma and Bali (1998) and Vaiyapuri et al. (1998).

Table 1
Growth components of rice (flowering stage) as influenced by stubble management practices

Treatments	Plant height (cm)	No. of tillers hill^{-1}	LAI	DMP (kg ha^{-1})
T_1	77.75	8.64	5.46	8722
T_2	73.16	7.45	5.21	8527
T_3	69.27	6.96	5.01	8282
T_4	67.93	6.34	4.61	8176
T_5	79.38	9.39	5.73	9193
T_6	74.69	7.89	5.28	8611
T_7	75.62	8.96	5.62	9141
T_8	68.17	6.71	4.84	8343
T_9	75.39	8.12	5.35	8638
T_{10}	66.27	5.83	3.96	6447
CD (P =0.05)	4.72	0.50	0.33	666

The stubble management practices exerted a significant influence on the yield attributes, namely number of productive tillers hill^{-1}, panicle length, total number of grains panicle^{-1}, number of filled grains panicle^{-1} and grain yield of rice.

Yield components and grain yield

Inoculation of *Trichoderma viride* at the time of stubble incorporation, followed by the application of 120 kg N ha^{-1} in 4 splits (T_5) gave maximum values of yield attributes and grain yield (Table 2), but was on par with the application of 90 kg N ha^{-1} in 4 splits + *Trichoderma viride* (T_7). The increase in the productive tillers hill^{-1} and the grain yield may be due to the greater number of early-formed tillers under the uninterrupted supply of N due to the mineralization of the organic source, i.e. stubble and straw. Similarly, the availability of adequate N during the later growth period may have promoted the translocation of assimilates to the sink, thus increasing the length of the panicles with a higher number of filled grains and thereby greater grain yield. Similar findings were also reported by Udayasoorian et al. (1997).

However, the influence of straw management practices on the test weight was not significant, because the 1000-grain weight is a stable and mostly genetically governed character, as reported by Yoshida (1981).

It can be concluded from the study that sprinkling *Trichoderma viride* at a rate of 1 kg^{-1} of stubble during stubble incorporation (6.25 t ha^{-1}), combined with the application of 120 kg N in 4 splits (25% each at basal, 15, 30 and 45 days after transplanting) was found to be the best stubble management practice for obtaining a better grain yield in second season rice in a rice-rice cropping system.

Table 2
Yield components and grain yield as influenced by the stubble management practice in rice

Treatments	Productive tillers hill^{-1}	Panicle length (cm)	Total grains panicle^{-1}	Filled grains panicle^{-1}	1000-grain weight (g)	Grain yield (kg ha^{-1})
T_1	6.37	21.54	108	79	20.61	5190
T_2	6.26	20.33	84	70	20.56	5050
T_3	5.67	20.19	89	68	20.54	4900
T_4	5.07	19.71	79	67	20.51	4685
T_5	7.19	21.66	109	83	20.64	5460
T_6	6.23	20.89	89	74	20.58	5070
T_7	6.89	21.57	107	82	20.63	5447
T_8	5.54	20.13	86	68	20.52	4850
T_9	6.26	21.43	97	77	20.58	5130
T_{10}	4.89	18.82	82	63	20.47	3840
CD (P =0.05)	0.39	1.34	6.0	5.0	NS	324

NS = non-significant

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Book review

GEORGE P. RÉDEI: *Encyclopedic Dictionary of Genetics, Genomics, and Proteomics.* 2nd Edition, 1392 pages, A John Wiley and Sons, Inc. Publication, Hoboken, NJ, USA 2003

There are more scientific books concerned with biology on the market now than at any time I can remember. Obviously, neither libraries nor individuals can afford to buy all the interesting titles. Hard decisions must be made and only books with extended and general value Must be chosen. Some may be highly specialized and useful only for ongoing research, others may assist in teaching and studying for advanced classes. Some books also offer information for professionals in various applied fields such as environmental and conservation sciences, plant and animal breeding, human or veterinary medicine, law or social work, or may just address the interest of the educated public. Biology is everywhere.

The question may arise of whether it is worth reading hard-copy books in the new age when so many databases are within reach on the Internet? Many libraries also provide access to numerous electronic journals. Would these not satisfy all our needs? Sadly, I must give a categorically No for several reasons. Even the best Internet journal/abstract resources are not selective, and do not evaluate or explain the individual entries. This can be illustrated with a single example. On February 7, 2004, I found 2640 entries in PubMed and 85 entries in Agricola for the term proteomics. More traditional areas are represented even more frequently in each of these databases. Since I do research, teach classes, advise students, write applications and reports and even have a family, it is impossible to cope with all my needs without good books.

Biology has undergone enormous changes in recent years. What appeared to be front-line knowledge only a few years ago, today requires thorough refurbishing just to stay afloat or barely keep abreast. Rédei mentions a now ten-year-old comment by the since Nobel laureate Sydney Brenner: "...genetics will disappear as a separate science, because in the 21st century everything in biology will be gene-based, and every biologist will be a geneticist."

This quote sets the tone and goal of this encyclopedic dictionary, which is more an encyclopedia than a dictionary, not by choice but by necessity. The stated goal of this book is the facilitation of communication and understanding across the wide range of biology. In this book, concepts are not just defined but explained in sufficient detail and the current references facilitate further reading if additional information is desired. The emphasis is on recent theoretical advances, new concepts, terms and their applications. Obviously no single book can include everything, yet it is hard to find a topic that this encyclopedia does not cover. Relatively few entries exceed a couple of thousand words, making it much faster to find the specific concept or term of interest. The ample cross-references between the entries completely compensate for the brevity of the entries. This single-author book is practically free of redundancy and is compact in size (compared with some journals, which publish tens of thousands of pages annually) but not in depth of information. Errors are minimal and even these are publishing errors. Many of the topics covered are not to be found in any other single book, including encyclopedias, dictionaries or glossaries. Since the publication of the first edition, the author has steadily updated and improved on the topics. He has added many new concepts, illustrations, references and database addresses. He has greatly expanded the cross-references between the entries. The 2nd edition contains more information, and more than twice as many illustrations than the first edition. A new feature is the predominantly current, over 7000 bibliographical references to journal articles. The bibliographies may help to locate additional key and classical papers. The General References at the end of the work include a list of about 2000 current books, many published in 2003. Colour figures have been added in a separate section in the centre of the book. At the end of each letter file there are some light-hearted historical vignettes.

The vision of biology today is not less than the complete understanding of how genes, cells and organisms are built, how they function metabolically and developmentally, how they operate in systems and how they have evolved. This one-volume encyclopedia does remarkably well to motivate and aid in this exciting endeavour.

J. M. KISS

Acta Agronomica Hungarica, 52(1), pp. 111–112 (2004)

Obituary

PÁL KOZMA
(1920–2004)

Pál Kozma, a scientist famous throughout Europe for his work on vines, was born into a poor peasant family in the small village of Gyulaháza in Szabolcs-Szatmár-Bereg County in Eastern Hungary on 11 July 1920. Despite his thirst for knowledge, he was obliged to interrupt his studies on several occasions due to the poverty of his family, and it was not until 1947 that he finally graduated from the University of Agriculture with a first class honours degree in agriculture, specialising in horticulture and vine-growing. The following year he obtained his teaching diploma, again with first-class honours.

In 1947 he started work as an assistant inspector of viticulture in Tarcal, later moving to the Technical College for Horticulture and Viticulture in Miklóstelep, where he was employed as a teacher and viticulture inspector. From 1949 onwards he worked in the Department of Viticulture at the Faculty of Horticulture and Viticulture of the University of Agricultural Sciences, filling the post of Head of Department from 1960 until he retired in 1990. From 1962–1965 he was Vice-Rector of the University, followed by six years as Rector from 1965–1971.

The basic and applied research he carried out from 1948 onwards gave a new direction to viticulture. His field of research included the flowering biology of the vine (flower morphology, histology, divergence and evolution of flower types, special types of fertilisation and grape formation in various flower types, light and electron microscope studies on morphological traits), vine breeding through selection and crossing (intra- and interspecific hybrids of white and red wine grapes and table grape varieties), leaf analysis for the study of the organic and mineral metabolism of vines and the diagnosis of optimum nutrient supplies, transpiration, the physiological effects of cultivation and pruning methods, the physiology of vine branches, improved technologies for the cultivation of table grapes, and the history of viticulture.

In addition to the success he achieved in scientific research, he was also an excellent teacher. His students left the university with a high standard of knowledge and many of them distinguished themselves in later life. In recognition of his achievements he was given many awards, including the State Prize in 1975 and the Order of the Hungarian Republic in 1990. He received a prize from the publishers for his books entitled "Table Grapes" in 1962 and "Vines and Their Cultivation I–II" in 1994. He also received a number of international awards, including the OIV Prize (1964, 1994), the Humboldt Memorial Plaque (1968) and the Hegel Medal, Berlin (1970). He was a member of the Editorial Committee of Acta Agronomica Hungarica from 1967 to 1994 and Chief Editor from 1995 to 2000.

Those who were privileged to know Pál Kozma found him to be a good-humoured and extremely well-informed man, with an enormous thirst for new knowledge and the determination which had stood him in good stead in his rise from the depths of poverty to the heights of an academic career. He was not only highly intelligent, but also extremely hard-working, never allowing difficulties to hinder him in his quest for knowledge. He will be sadly missed, but his influence will remain with us in his books and in the work of the experts he trained so well.

Z. CSOMA

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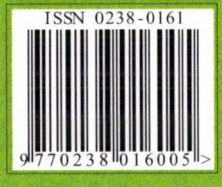

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STRESS TOLERANCE RESPONSES OF TWO COTTON CULTIVARS EXPOSED TO ULTRAVIOLET-A (366 nm) RADIATION: PHOTOSYNTHETIC PERFORMANCE AND CHEMICAL CONSTITUENTS

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The stress tolerance responses of two Egyptian cotton cultivars (Giza 45 and 86) exposed to various doses (40, 80, 160 and 320 min) of artificial ultraviolet-A (366 nm) radiation were investigated. The seed germination of Giza 86 was promoted at 40 min, but substantially inhibited at 80 and 160 min and completely suppressed at 320 min. However, the seed germination of Giza 45 was progressively inhibited by UV-A exposure and ceased at 160 min, so doses of 40 and 80 min were selected for further studies. In contrast to seed germination, the seedling growth of Giza 86 was negatively affected at 40 min. UV-A stress induced a great reduction in the leaf carbohydrates as well as in the viability and dry mass production of the shoots of both cultivars, but the response was comparatively higher in Giza 45. It also decreased the chorophyll (Chl) and carotenoid contents, coupled with an increase in the Chl *a/b* ratio, diminished the Hill reaction activity, and quenched the Chl *a* fluorescence both in the presence and absence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, suggesting an inhibitory effect on the water-splitting system (donor side) as well as on the electron transport from the primary to the secondary acceptors of PSII (acceptor side). These changes reflect a disturbance in the structure, composition and function of the photosynthetic apparatus as well as the sensitivity of PSII to UV-A stress. Nucleic acids (DNA and RNA) were markedly damaged by exposure to UV-A for 80 min, while both cultivars developed adaptive mechanisms for damage moderation. These mechanisms involved increasing the levels of flavonoids, total lipids and total soluble proteins as well as having smaller, thicker leaf blades. Since Giza 86 showed a comparatively higher level of adaptation, it tolerates UV-A stress better than Giza 45.

Abbreviations: Car, carotenoids; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenol indophenol; DNA, deoxyribonucleic acid; d.m., dry mass; f.m., fresh mass; PSII, photosystem II; RNA, ribonucleic acid; TSP, total soluble proteins; UV-AR, ultraviolet-A (366 nm) radiation.

Key words: cotton cultivar, ultraviolet-A radiation, photosynthetic performance, stress tolerance

Introduction

Since the discovery of the ultraviolet (UV) wavelength band (200–390 nm), extensive studies have been made to investigate its characteristics as well as its possible effects on living organisms. These studies have classified this band of radiation into three subbands: 1) UV-C (200–280 nm), which is extremely harmful to living organisms, 2) UV-B (280–320 nm), which is of particular interest, because the depletion of the ozone (O_3) layer mainly allows this band of radiation to reach the earth's surface, and 3) UV-A (320–390 nm) which represents the least hazardous part of UV radiation (Murthy and Rajagopal, 1995). The wavelength of the solar electromagnetic radiation

reaching the earth's surface is not below 290 nm, due to the presence of the O_3 layer in the stratosphere, which characteristically absorbs the shorter wavelengths (Moorthy and Kathiresan, 1998). Hence UV radiation reaching the earth's surface consists of UV-A and UV-B, which may be increased due to the thinning and depletion of the O_3 layer, linked to the industrial emission of chlorofluorocarbons (Fraser and Prather, 1999; Feng et al., 2003).

Over 90% of the UV radiation (UV-R) reaching the earth's surface is UV-A, while the rest (10%) includes UV-B (Hamada, 2002; Lud et al., 2002). Nevertheless, the largest volume of work has been done on responses to UV-BR, which exerts substantial negative effects on most physiological processes in plants (Friso et al., 1994; Shishkin and Ivanishchev, 1997; Abd-El-Kareem, 1999; Lud et al., 2002; Feng et al., 2003) due to its absorption by important biological macromolecules, such as proteins and nucleic acids (Jansen et al., 1998). However, changes in seed germination and certain physiological traits of chicory seedlings have also been reported after varying doses of UV-CR (Hamada, 2002).

UV radiation can penetrate through plant leaves and be absorbed by chromophores, associated with the photosynthetic apparatus, and/or by genes and gene products (Robberecht et al., 1980; Joshi et al., 1994; Allen et al., 1998). Cellular components that can directly absorb UV-R include nucleic acids, proteins, lipids and quinones (Jordan, 1996). Water-soluble phenolic pigments, such as flavonoids, can strongly absorb UV-R whilst not absorbing photosynthetically active radiations (Allen et al., 1998). Therefore, leaf penetration by UV-R may be negatively correlated with such compounds (UV-absorbing compounds) as well as with the leaf thickness. UV stress can potentially impair photophosphorylation, CO_2 fixation and/or stomatal control of the CO_2 supply (Fiscus and Booker, 1995; Allen et al., 1998), hence, it may damage the overall photosynthesis process and consequently the plant growth and productivity. In this regard, PSII has been reported to be the most sensitive component of the thylakoid membrane, on exposure to UV-R (Hirosawa and Miyachi, 1983; Panagopaulos et al., 1990; Friso et al., 1994; Fiscus and Booker, 1995; Allen et al., 1998; Feng et al., 2003).

Cotton is preferably grown in mid-latitudes. Due to its occurrence in open habitats, it is liable to be affected by UV-AR as well as by the depletion of the O_3 layer. UV-AR is an environmental stress factor experienced by plant leaves (Joshi et al., 1997). Despite the higher percentage of UV-AR, relatively little work has been done on plant responses to it (Hirosawa and Miyachi, 1983; Scherer et al., 1988; Joshi et al., 1991, 1994, 1997) and no work has reported on the cultivar or tolerance responses to such stress. The present study was conducted to test whether two Egyptian cotton cultivars, Giza 45 and Giza 86, differed in their sensitivity to UV-A (366 nm) radiation. Germination, photosynthetic responses and the levels of nucleic acids and some metabolites were determined after UV-A treatment. Foliar flavonoids, leaf area and leaf thickness were also examined to help in estimating the relative contribution to plant tolerance of UV-AR.

Materials and methods

Plant material, growth conditions and treatments

Seeds of cotton (*Gossypium barbadense* L.) cultivars Giza 45 and 86 obtained from the Cotton Research Institute (ARC, Giza, Egypt) were surface sterilized in 2% Na-hypochlorite for 10 min, then rinsed with sterile distilled water. The sterilized seeds of each cultivar were soaked in water for 12 h, then used in Petri-dish and pot experiments.

In the Petri-dish experiment, the soaked seeds were irradiated with UV-A from a UV-lamp (model UV GL-58, Mineral light lamp, Multiband UV-254/366 nm, 215–250 volt, 50/60 Hz, 0.12 AMPS, San Gabriel, CA 912778, USA) at a wavelength of 366 nm for 0, 40, 80, 160 and 320 min. The irradiated seeds were regularly distributed in Petri-dishes (10 cm diameter) on moist filter papers (3 cm^3 distilled water per paper), and moistened with half-strength Hoagland nutrient solution (Hoagland and Arnon, 1950) for 10 days. The seeds (20 per dish) were germinated in the dark, by covering the Petri-dishes with black bags for 5 days, then under laboratory conditions for another 5 days with a 12 h photoperiod (with a light intensity of 120–140 μmol m^{-2} s^{-1}), at day/night temperatures of 30/18°C and 65–75% relative humidity. The germination percentage was determined on the tenth day after sowing. The results allowed irradiation doses of 40 and 80 min to be chosen for further studies, as described below.

In the pot experiment, the soaked seeds were sown in plastic pots (16 cm diameter, 12 cm height) filled with clay loam soil (2 kg pot^{-1}, 12 seeds per pot). On the third day after emergence (i.e. 13 days after sowing, DAS) the seedlings were thinned to six per pot, then the pots were randomly divided into three groups. The seedlings in the first group were not irradiated (control), whereas those in the second and third groups were directly irradiated (in the dark, at a distance of 20 cm) with UV-A (366 nm) for 40 and 80 min, respectively. The seedlings were re-irradiated at 4-day intervals until 15 days after emergence (i.e. 25 DAS). The sowing date was April 14, 2002 and the experiment was conducted under the laboratory conditions described above. The pots were irrigated with full-strength Hoagland nutrient solution, to slightly less than the field capacity level, whenever required. At 25 days old, the plant samples were randomly selected and separated into roots and shoots, after which the leaves and shoots were used for further analyses, as described below.

Foliar pigments

Leaf chlorophyll (Chl a and b) and carotenoids (Car) were extracted with 80% acetone [0.5 g f.m. (10 cm^3 acetone)$^{-1}$], then quantified according to Lichtenthaler and Wellburn (1983). Flavonoids were extracted with 70% ethanol [0.5 g f.m. (10 cm^3 ethanol)$^{-1}$] (Harborne, 1984), and spectrophotometrically determined (relative units, A 305 nm) following the method of Feng et al. (2003).

Photosynthetic (Hill reaction) activity

The Photosystem II (PSII) activity of chloroplasts isolated from the leaves of fresh seedlings, expressed as the electron transport rate, was determined using 2,6-dichlorophenol indophenol (DCPIP) as electron acceptor (Biswal and Mohanty, 1976). Chloroplasts were isolated in the cold, as described by Osman and El-Shintinawy (1988). The concentration of Chl *a*+*b* in the supernatant was determined using the equation of Arnon (1949). For measuring the PSII activity, an assay sample was prepared by mixing 1.6 cm^3 of 10 mM DCPIP (dissolved in 96% ethanol) with 50 μg Chl, making up the volume to 3 cm^3 with reaction buffer. The sample was irradiated (at right angles) with red actinic radiation (300 W m^{-2}, 10 min) provided by a slide projector. The DCPIP photoreduction was spectrophotometrically assayed according to Ebrahim and Aly (2004).

Chl a fluorescence emission spectra

These spectra were measured in the absence and presence of 3-(3,4-dichlorophenyl)-1-1-dimethylurea (DCMU) at room temperature (30±2°C) according to Tripathy et al. (1981) with some modifications. The blue actinic radiation was switched on and focused on the sample cuvette by a spectrofluorometer (model *510,* Schimadzu, Japan). Chloroplast isolation and Chl

determination were carried out as described above. Isolated chloroplasts were suspended in the reaction buffer, then transferred to the sample cuvette. The assay volume was 3 cm^3 and the Chl concentration was 5 g m^{-3}. All the samples were dark-adapted for 15 min prior to measurements. DCMU (10 μM) was incubated with the assay volume (in the dark) in the measuring cuvette for 2 min. Thereafter, the samples were excited by blue actinic radiation (460 nm). The emission kinetics (signals) produced were photomultiplied and recorded by means of a recorder.

Dry leaf measurements

Fresh leaves were dried in an aerated oven at 70°C to constant weight. Nucleic acids were extracted and determined following the method described by Bassett et al. (1988). Proteins and carbohydrates were extracted in borate buffer pH 8 [0.1 g d.m. (10 cm^3 buffer)$^{-1}$]. Soluble proteins were determined according to the method of Lowry et al. (1951). Total carbohydrates were determined by the methods of Naguib (1963; 1964). Lipids were extracted in a chloroform-methanol mixture (2:1 v/v) for 24 h at 4°C, then the extract was evaporated to dryness in a tared vial. The lipid residue in the vial was dried at 100°C, cooled in a desiccator and weighed (Osborne and Voogt, 1978).

Fresh leaf and shoot measurements

Leaf area and thickness were determined according to Rhodes and Bloodworth (1964). Shoot strength was calculated as described by Ebrahim (2003) and scored as follows: (a) negative growth = 1, (b) below-average growth = 2, (c) average growth = 3, (d) above-average growth = 4, and (e) excellent growth = 5.

Dry shoot measurements

Fresh shoots were dried in an aerated oven at 70°C to constant weight, then shoot biomass was determined in terms of dry mass (weight) per plant.

Statistical analysis

The data were averaged and statistically analysed using two-way analysis of variance (ANOVA). The least significant difference at the 0.05 level was used to compare means indirectly by the multiple range test of Duncan (1955) or directly according to Steel and Torrie (1980).

Results

The most important consideration for successful cotton production is the selection of a cultivar capable of maximizing the utilization of available resources under stress conditions. Therefore, this study aimed at determining the genotypic stability of two Egyptian cotton cultivars (Giza 45 and 86) stressed by UV-AR. A preliminary experiment showed that irradiating seeds with UV-A (366 nm for 0, 40, 80, 160 and 320 min) progressively reduced the percentage of seed germination in Giza 45 (Table 1). In contrast, the seed germination of Giza 86 was stimulated at the lowest dose (40 min), but evidently inhibited by the moderate and severe doses (80 and 160 min) and completely suppressed by the highest dose (320 min) of UV-AR. Although the seed germination of Giza 45 was completely retarded by a dose of 160 min, 46% of the seeds of Giza 86 were germinated. Despite the non-significant differences between the germination rates of Giza 86 and 45 at 80 and 40 min, the axes grew more rapidly and their growth was more uniform in Giza 86 compared with Giza 45 (data not shown). These results demonstrated the greater genotypic stability and stress tolerance of the cultivar Giza 86. They also allowed irradiation doses of 40 and 80 min to be selected for further studies.

UV-A irradiance caused significant and progressive reductions in the Chl and Car contents, as well as in the PSII activity, expressed as DCPIP photoreduction, of isolated chloroplasts of both cultivars (Table 2). Conversely, the Chl *a/b* ratio was increased by exposure to UV-A, suggesting that Chl *b* is more sensitive to UV-A than Chl *a*. Although both cultivars showed similar responses to UV-A irradiance, the changes were comparatively greater in Giza 45. Exposure to UV-A for 80 min led to reductions of 9.9 and 5.4% in Chl *a*, 22.8 and 17.9% in Chl *b*, 38.5 and 29.6% in Car, and 24.3 and 19.2% in PSII activity in Giza 45 and Giza 86, respectively. To determine the site of the inhibitory effect of UV-A on PSII activity, Chl *a* fluorescence emission spectra were studied in the presence and absence of DCMU (Fig. 1), which blocks the electron flow from the primary (Q_A) to the secondary (Q_B) quinone acceptors and thus chemically isolates PSII from PSI (Cao and Govindjee, 1990). On excitation at 460 nm, isolated chloroplasts showed an emission peak at 680–685 nm, from the Chl *a* of PSII. Both in the presence and absence of DCMU, UV-A irradiance progressively quenched the fluorescence emission, with shifts in the peaks observed more drastically in Giza 45. However, in all treatments, Chl *a* fluorescence displayed higher intensities in the presence than in the absence of DCMU (Fig. 1).

Table 1

Effect of UV-AR on germination (%) of two cultivars of cotton. Seeds were germinated in Petri-dishes for 10 days after sowing, under laboratory conditions

UV dose (min)	Giza 45	Giza 86
0	76 b*	78 b
40	57 d	88 a
80	38 e	61 c
160	0.0 f	56 d
320	0.0 f	0.0 f

*Mean values followed by the same letter do not differ significantly ($P \leq 0.05$)

Table 2

Effect of UV-A radiation on photosynthetic pigment contents [mg (g d.m.)$^{-1}$], Chl A/B ratio and photosystem II (PSII) activity [μmol DCPIP reduced (mg Chl)$^{-1}$ h^{-1}] in leaves of 25-day-old cotton plants

Cultivar	UV dose (min)	Chl A	Chl B	Chl A/B ratio	Car.	PSII activity
Giza 45	0	3.64	1.40	2.60	2.6	74
	40	3.51	1.20	2.92	2.3	66
	80	3.28	1.08	3.04	1.6	56
Giza 86	0	3.90	1.40	2.78	2.7	78
	40	3.80	1.30	2.92	2.5	71
	80	3.69	1.15	3.21	1.9	63
Factor		LSD at 5% level				
Cultivar		*	*	–	*	*
UV dose		0.14	0.05	–	0.08	4.16
Cultivar × UV dose		0.21	0.07	–	0.12	5.68

* = significant difference.

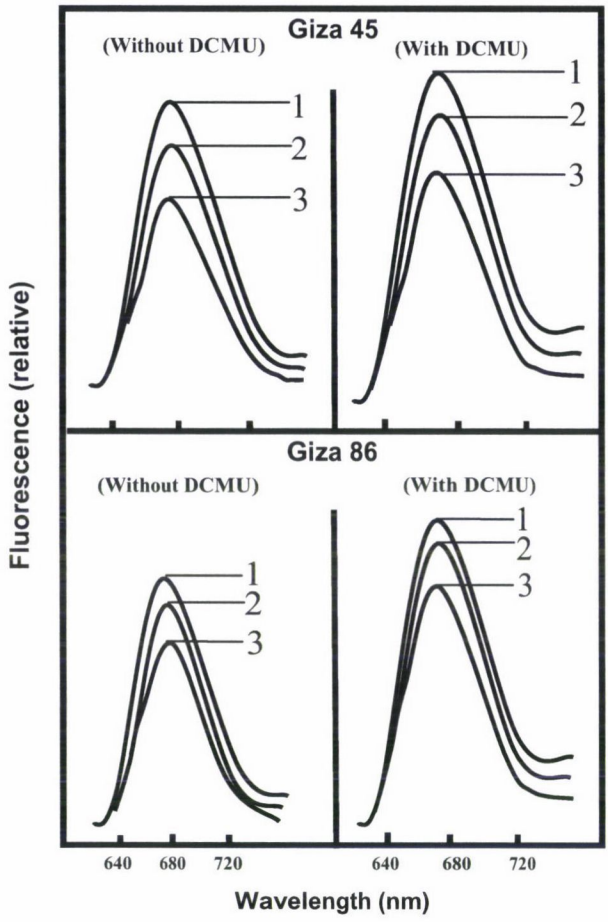

Fig. 1. Chlorophyll a fluorescence emission spectra of chloroplasts isolated from leaves of 25-day-old plants of two cultivars (Giza 45 and Giza 86) previously exposed to different doses of UV-AR. 1 = control, 2 = UV-AR for 40 min and 3 = UV-AR for 80 min

In comparison to the control, UV-A stress caused a significant decrease in the foliar contents of DNA and RNA, but a contrary trend was noticed in the case of flavonoids, total lipids and TSP contents in both cotton cultivars (Table 3). In addition, the cultivar Giza 86 had higher nucleic acid, flavonoid, total lipid and TSP contents than Giza 45.

Exposing plants to UV-A inhibited the growth and development of both cultivars (Table 4). Plants of Giza 45 appeared to be more stressed by UV-A irradiance than those of Giza 86. In both cultivars, UV-A substantially increased the leaf thickness. Conversely, it reduced the leaf area and carbohydrate accumulation by the leaves as well as the strength (viability and vigour) and dry mass production of the shoots, and these reductions were aggravated at 80 min, particularly in Giza 45. Therefore, cultivar Giza 86 is judged to be more tolerant to UV-A stress than Giza 45.

Table 3

Effect of UV-AR on nucleic acids, flavonoid content (relative units, A 305 nm), and lipid and total-soluble protein (TSP) contents [mg (g d.m.)$^{-1}$] of leaves of two cultivars of 25-day-old cotton plants

Cultivar	UV dose (min)	DNA	RNA	Flav.	Tot. lipids	TSP
Giza 45	0	4.4	3.8	1.2	81	107
	40	4.2	3.4	1.3	90	118
	80	4.0	2.7	1.8	111	134
Giza 86	0	4.8	3.9	1.3	83	114
	40	4.5	3.7	1.6	93	130
	80	4.2	3.4	2.0	117	150
Factor		LSD at 5% level				
Cultivar		**	**	*	*	*
UV dose		0.29	0.17	0.12	7.4	8.12
Cultivar × UV dose		0.38	0.25	0.17	9.8	11.48

* = significant difference, ** = highly significant difference

Table 4

Effect of UV-AR on area (cm^2), thickness (mm) and carbohydrate content [mg (g d.m.)$^{-1}$] of leaves, and strength (str., relative units) and dry mass [g (plant)$^{-1}$] of shoots of 25-day-old plants of two cotton cultivars

Cultivar	UV dose (min)	Leaf area	Leaf thick.	Tot. carb.	Shoot str.	Shoot d.m.
Giza 45	0	12.4	0.80	484	3.0	2.2
	40	11.1	0.90	450	2.6	1.7
	80	8.7	1.02	391	2.0	0.9
Giza 86	0	11.5	0.87	488	4.0	2.5
	40	9.0	0.99	470	3.5	2.1
	80	6.9	1.19	410	2.5	1.6
Factor		LSD at 5% level				
Cultivar		**	*	*	**	**
UV dose		0.81	0.07	22.1	0.19	0.14
Cultivar × UV dose		1.14	0.09	NS	0.26	0.19

* = significant difference, ** = highly significant difference; NS: non significant

Discussion

The viability and photosynthetic performance of cotton seedlings and the accumulation of certain chemical constituents in the leaves could be considered as integral indexes for stress tolerance response. These characteristics were thus used to assess the general effect of UV-AR (366 nm) on the germination, growth and development of two Egyptian cotton cultivars (Giza 45 and Giza 86).

The effect of various doses of UV-AR on seed germination is an important factor in predicting the possible response of cotton seedlings and adult plants to stress caused by UV-A irradiation. Therefore, the influence of UV-A doses on seed germination was first examined. Seed exposure to UV-A was shown to lead to cultivar- and dose-dependent changes in seed germination, in

agreement with results recorded by Shishkin and Ivanishchev (1997) and Hamada (2002). This was associated with the inhibition or stimulation of some early metabolic processes related to seed germination. Therefore, these processes are believed to be progressively inhibited by UV-A doses in both cultivars. Although the mechanism of these changes is still not clear and has been poorly investigated, a change in the activity of the enzymes involved in the mobilization of stored compounds within the seeds appears to be implicated. The greater genotypic stability of Giza 86 compared with Giza 45 may be interpreted as the better adaptive response of seedlings of Giza 86, which alleviates the adverse effects of UV-A stress. Further investigations on the metabolic processes related to seed germination, respiration rate and protein and isozyme patterns may support the interpretation suggested in the present work.

UV-AR is an environmental stress factor experienced by plant leaves (Joshi et al., 1997). The deleterious effects of UV-R are reported to be partitioned between damage to the photosynthetic machinery and damage to the plant genome (Fiscus and Booker, 1999; Murthy and Rajagopal, 1995; Jansen et al., 1998; Moorthy and Kathiresan, 1998). The decrease in Chl and Car contents caused by all doses of UV-AR is in agreement with the results obtained by other authors (e.g. Murthy and Rajagopal, 1995; Hamada, 2002; Feng et al., 2003), who attributed this response to changes in pigment biosynthesis and/or degradation, but the exact mechanisms for such changes are not yet clear. The increase in the Chl *a/b* ratio in irradiated seedlings could be mainly ascribed to the greater destruction of Chl *b* than Chl *a* (see Table 2). This may lead to changes in the composition and structure of the Chl *a/b* protein complex (Feng et al., 2003) which, in turn, may influence chloroplast development (El-Shintinawy et al., 2004). The comparative decrease in PSII activity in Giza 45 and during UV-A treatment could be due to: 1) a decline in energy transfer from the light harvesting complex to the reaction centre, 2) the inability of the reaction centre to accept photons as a result of the altered architecture of the PSII complex, and/or 3) a dramatic decrease in the electron flow from the Q_A to Q_B quinone acceptors of PSII (Cao and Govindjee, 1990; El-Shintinawy et al., 2004). To examine the above possibilities, Chl *a* fluorescence emission spectra were investigated in the presence and absence of DCMU (see Fig. 1). In the absence of DCMU, the quenching of Chl *a* fluorescence reflects inefficient energy transfer from the light harvesting complex to the reaction centre of PSII (Cao and Govindjee, 1990), probably as a result of structural alterations in the PSII complex. This quenching was partly mitigated by DCMU, suggesting the inhibitory effect of UV-A irradiance on electron transport from Q_A to Q_B. Therefore, these results confirm the inhibitory effect of UV-AR on both the donor and the acceptor sides in isolated chloroplasts. They also demonstrate the impairment of the photochemical efficiency of the PSII protein complex by UV-A irradiance. Since this impairment was comparatively lower in Giza 86, Giza 45 showed more sensitivity to UV-A stress than Giza 86.

Since UV stress damages DNA and RNA as well as overall photosynthesis (Fiscus and Booker, 1995; Jansen et al., 1998), plants are able to develop repair and adaptive mechanisms to reduce this damage (Jansen et al., 1998; Lud et al., 2002). These mechanisms include increasing the levels of UV-absorbing compounds (e.g. flavonoids, total lipids and TSP) as well as having smaller, thicker leaf blades (Fiscus and Booker, 1995; Murthy and Rajagopal, 1995; Feng et al., 2003). Hence, the reduction in DNA and RNA revealed in the present study reflects damage to the plant genome due to the absorption of UV-A by nucleic acids (Jansen et al., 1998). However, the comparatively lower extent of damage in Giza 86 could be due to the relatively higher capacity for replacing the damaged sequences of nucleic acids with sequences newly synthesized from complementary strands in a process called excision repair (Fiscus and Booker, 1995), but further studies on DNA sequences will be needed to support this interpretation. The aggravated damage to nucleic acids by UV-A irradiance for 80 min may be due to the stimulation of their breakdown through enhancing the activities of DNAase and RNAase. The increase in flavonoids, total lipids and TSP levels under UV stress was shown by other authors (Scherer et al., 1988; Fiscus and Booker, 1995; Moorthy and Kathiresan, 1998; Feng et al., 2003), who ascribed this response to the plant's ability to develop protective mechanisms against such stress. In this respect, the enhanced production of flavonoids, total lipids and TSP could be due to the greater production of the genes coding their biosynthetic enzymes (Fiscus and Booker, 1995; Murthy and Rajagopal, 1995). Cultivars exhibiting higher flavonoid content coupled with smaller leaf area and thicker leaf blades were comparatively more tolerant to UV stress (Feng et al., 2003). In the present work, the positive correlation between the levels of UV-absorbing compounds and the magnitude of UV-A stress confirmed the role of such compounds in damage moderation and hence in plant tolerance to UV stress. The final important protective mechanism may be the synthesis of specific proteins, i. e. stress proteins similarly to the heat shock proteins synthesized in the case of heat stress. Further investigations at the protein and isozyme level and on the thylakoid membrane structure and the enzymatic antioxidant protective system will be required to support this interpretation.

Since photosynthesis is the engine driving overall plant growth and productivity, the biomass production by the shoots of test cotton plants showed a positive correlation with the foliar carbohydrate content, which, in turn, seemed to be dependent upon the overall photosynthesis process. The reduction in the foliar carbohydrate content and the inhibition of shoot growth under UV-A stress were reported by other authors (Hirosawa and Miyachi, 1983; Joshi et al., 1991; 1994; 1997), who attributed this response to damage to the photosynthetic machinery, cell division and/or the plant genome. The present data, recorded in Tables 2 and 3, support this interpretation. The changes in the leaf area and thickness of both cultivars under UV-A stress could be seen as a stress tolerance

response moderating the damage levels. The greater stress tolerance of the cultivar Giza 86, compared with Giza 45, may be linked with the genotypic stability of the cultivars. Such tolerance can be explained on the basis of the relative ability of the test cultivars to develop repair mechanisms adequate for damage moderation. These mechanisms were reported to involve a decrease in leaf area and an increase in leaf thickness and UV-absorbing compounds (Fiscus and Booker, 1995; Allen et al., 1998). Therefore, the data recorded in Tables 3 and 4 support this interpretation and explain why cultivar Giza 86 seemed more tolerant to UV-A stress than Giza 45.

In conclusion, cotton responses to UV-A irradiance are quite variable and seemed to be cultivar- and dose-dependent. Deleterious UV-A effects may be largely partitioned between damage to the photosynthetic machinery and damage to the plant genome. Almost every facet of the photosynthetic machinery may be directly damaged by severe doses of UV-A, but the electron transport mediated by PSII appeared to be the most sensitive, where the damage seemed to involve all parts of the PSII complex from the Mn binding site to the plastoquinone acceptor sites on the opposite surface of the thylakoid membrane. In addition, direct damage to DNA and RNA has been shown to result from exposure to high doses of UV-AR. However, cotton plants appear to possess repair mechanisms adequate to deal with the damage levels. These mechanisms involved the ability to increase UV-absorbing compounds (flavonoids, total lipids and total soluble proteins) as well as the ability to decrease the leaf area and to increase the leaf thickness. These changes may help plants to tolerate the stress induced by UV-AR. Seedlings of Giza 86 attained a comparatively lower leaf area, greater leaf thickness and higher levels of UV-absorbing compounds, so it appeared to be more tolerant to UV-A stress than Giza 45.

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POST-HARVEST CHARACTERISTICS OF CUT CARNATIONS AS THE RESULT OF CHEMICAL TREATMENTS

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Cut flowers of *Dianthus caryophyllus* L. cv. Asso were treated with 8-hydroxyquinoline sulphate (8-HQS) at 200 and 400 ppm with or without sucrose at 50 g l^{-1}, silver thiosulphate (STS) at 0.2 and 0.4 mM with or without sucrose at 50 g l^{-1}, and 1-methylcyclopropene (1-MCP) at 0.3, 0.5 and 0.7 g m^{-3} for 6 h to study the effect of these chemicals on post-harvest quality. 8-HQS treatments increased the vase life and the percentage loss of initial fresh weight compared to the control. In addition, the vase life was longer when sucrose was applied in combination with 8-HQS. The best treatment involved 400 ppm 8-HQS + 50 g l^{-1} sucrose. All the concentrations of STS prolonged the vase life and fresh mass compared to the control. The best treatment was STS at 0.4 mM with or without sucrose. All levels of 1-MCP prolonged the vase life and increased the fresh weight in comparison with the control. The best treatment in this respect was 1-MCP at 0.5 g m^{-3} for 6 h. The chlorophyll content (chl *a* and chl *b*) in the leaves was higher than the control in the best treatment of each chemical.

Key words: cut flowers, fresh weight, 8-HQS, 1-MCP, STS, vase life

Introduction

Carnations (*Dianthus caryophyllus* L.) are still one of the mainstays of the cut flower industry and are among the most popular florist crops, growing naturally in many parts of the world. Consequently, there is considerable competition between carnation producers (Larson, 1992; AIPH, 2003).

A major cause of the deterioration in cut flowers is the blockage of xylem vessels by microorganisms that accumulate in the vase solution or in the vessels themselves. When the stem is blocked, continuing transpiration by the leaves results in a net loss of water from the flower and stem tissues. For many years, floral preservatives have been acidified and have usually included biocides to inhibit bacterial proliferation (Nowak and Rudnicki, 1990). Hydroxyquinoline citrate (HQC) increased the vase life, as well as improving the fresh mass of cut carnation flowers compared to the control (Knee, 2000). Different authors reported that treatment with 8-HQS plus sucrose led to the prolongation of the vase life of various cut flowers (Patil et al., 2001; Dineshbabu et al., 2002; Hassan et al., 2003; Tar and Hassan, 2003).

Carnation flowers are sensitive to ethylene. Cut flowers produce small amounts of ethylene just after harvest, while there is a sharp increase in ethylene production a few days later. The deleterious effects of ethylene exposure include leaf yellowing, flower (or petal) drop, irregular opening and premature death (Nowak and Rudnicki, 1990).

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Since the 1970s, the best weapon against ethylene has been silver thiosulphate (STS), which can more than double the vase life of cut flowers. Menguc and Usta (1994) reported that STS + sucrose pretreatment had a positive effect on the vase life and petiole size of cut carnations. Celikel et al. (1995) found that STS pulsing prolonged the vase life of cut carnation flowers to 15.5 days compared to 6.8 days in the control. Altman and Solomos (1995) mentioned that flowers continuously treated with 0.2 mM STS exhibited no morphological or respiratory responses to any concentration of exogenous ethylene, whereas both a respiratory increase and irreversible petal wilting were observed in flowers pulsed with 0.5 mM STS. They also suggested that the interaction between silver ions and ethylene is competitive.

Because STS contains silver, which is now considered to be a potential environmental pollutant, there has been some restriction on its commercial use (Serek and Reid, 1993; Cross, 1996). Researchers have therefore been seeking alternative strategies, including the use of inhibitors of ethylene biosynthesis and inhibitors of ethylene binding, to prevent the undesirable post-harvest effects of ethylene. A new tool, 1-methylcyclopropene (1-MCP), has been added to the list of options for extending the vase life of cut flowers. 1-MCP, soon to be marketed under the trade name EthylBloc, was discovered by Dr. Sisler of North Carolina State University, Raleigh, North Carolina, United States. 1-MCP is a gas in its natural state (as is ethylene), which provides opportunities and challenges in commercial use. EthylBloc will come in powder form, which is added to water to release the gas. 1-MCP is a non-toxic inhibitor of ethylene action, acting as a competitive and irreversible inhibitor of the binding of ethylene to its receptor (Sisler et al., 1996). Even at very low concentrations it has been shown to eliminate the effects of ethylene on the abscission and wilting of many ornamental crops, such as carnation and rose (Serek et al., 1995; Sisler et al., 1996). The longevity of cut carnations pre-treated with 1-MCP increased, and 1-MCP protected carnation flowers against ethylene for several days, extending their vase life (Serek et al., 1995; Sisler and Serek, 2001). Hassan and Gerzson (2002) found that treatment with 1-MCP at 0.5 g m^{-3} for 6 h led to an increase in the vase life as well as minimizing the percentage loss of the initial weight of cut carnations.

The aim of this research was to study the post-harvest quality of cut carnations as affected by 8-HQS with or without sucrose, STS with or without sucrose and 1-MCP, and to study the efficacy of 1-MCP in this respect compared with the other chemicals.

Materials and methods

Plant material

Cut flowers of *Dianthus caryophyllus* L. cv. Asso, which produces large, white, standard flowers, were used in the experiment. The flowers were obtained from a commercial grower in Hungary at commercial maturity (half-open flowers) and brought to the laboratory of Budapest University of Economic Sciences and Public Administration after harvest. The lower leaves were removed and the flowering stems were trimmed to a uniform length of 50 cm.

Chemical treatments

8-HQS treatments

8-HQS was applied as a continuous treatment at concentrations of 200 and 400 ppm with or without sucrose at 50 g l^{-1}. The flowers were placed in glass vials containing 500 ml 8-HQS solution of the given concentration for the whole period of the experiment.

STS treatment

STS was prepared as described by Gorin et al. (1985). The cut flowers were treated with STS for 6 h at concentrations of 0.2 and 0.4 mM with or without sucrose at 50 g l^{-1}. After pulsing treatments the flowers were placed in glass vessels containing tap water till the end of the experiment.

1-MCP treatment

1-MCP (or EthylBloc) was obtained from the AgroFresh Inc. Rohm and Haas company. The flowers treated with EthylBloc were laid in a 118 × 28 × 44 cm box for the treatment. The box was sealed well with a plastic cover and the concentrations of 1-MCP were calculated as g m^{-3} (EthylBloc per cubic metre). The EthylBloc powder was weighed and placed in a test tube fixed with a self-adhesive tape to the inside wall of the box. Since a significant percentage of the 1-MCP is released immediately after the addition of hot water, the box was first sealed, and then hot water was injected into the test tube (just enough to cover the powder for each treatment). The concentrations used were 0.3, 0.5 and 0.7 g m^{-3} for 6 h. The treatment with 1-MCP was conducted at 15°C for all treatments. After the treatments the flowers were aerated and then placed into glass vials containing 500 ml tap water.

The control flowers were put into glass vessels containing 500 ml tap water for the whole period of the experiment.

Vase life determination

The longevity of cut flowers was determined in a vase life evaluation room with normal daylight at 20 ± 1°C and 80–90% RH. The visual rating of the flowers was evaluated on a scale from 1 to 4, where: 1 = entirely white flowers, 2 = initiation of darkening (wilting) in 20% of petals, 3 = darkening in 20–50% of petals, 4 = darkening in 50–100% of petals. The longevity of cut carnations was defined as the number of days of vase life required for 50% of the flowers to reach stage 2 or more advanced stages.

Fresh weight measurements

Fresh weight determinations were made just before the flowers were immersed into solution and were repeated on the day when the vase life of the control flowers was terminated. The flowers were taken out of the solution for as short a time as possible (20–30 s). The fresh weight of each flower was expressed relative to the initial weight to represent the % of fresh weight.

Chlorophyll determination

The chlorophyll content of the leaves was measured after extraction with acetone, as previously described by Dawood (1993), from samples of cut leaf segments (0.5 g) taken from the best treatment of each chemical on day 3 and on the day when the vase life of the control flowers was terminated. The samples were taken from the upper part of the stems. The chlorophyll content was calculated as mg g^{-1}.

Analysis of results

Three replications of five flowers per treatment were used in this experiment. The results were analysed using SPSS program Base 9 (SPSS, 1999). The analysis of variance (ANOVA) and the calculation of differences between means were performed using Duncan's multiple range test at the 0.05 level. The experiment was repeated at least twice.

Results

Effect of 8-HQS, STS and 1-MCP on vase life

Effect of 8-HQS

The results in Table 1 show that all the 8-HQS treatments prolonged the vase life of cut carnations compared to the control. The 8-HQS treatment increased the vase life with increasing concentration. Adding sucrose to all concentrations of 8-HQS gave longer vase life than 8-HQS treatments without sucrose. The best treatment was 400 ppm 8-HQS + 50 g l^{-1} sucrose, which gave a vase life of 19.3 days, compared to 8.3 days for the untreated control.

Effect of STS

All the concentrations of STS increased the vase life of cut carnations. There were no significant differences between the treatments with or without sucrose. The longest vase life was obtained by treatment with STS at 0.4 mM combined with sucrose, which resulted in 23.7 days, compared to 8.3 days for the control (Table 1).

Effect of 1-MCP

As indicated by the data in Table 1 all levels of 1-MCP prolonged the vase life of cut carnations. The best treatment in this respect was 1-MCP at 0.5 g m^{-3} for 6 h, which gave a value of 17.3 days, compared to the untreated control (8.3 days).

Fresh weight as a percentage of initial weight

The influence of 8-HQS on the fresh weight % of cut carnations exhibited a positive correlation with the concentration of 8-HQS and with the addition of sucrose to all levels of 8-HQS. The highest fresh weight % was obtained with 400 ppm 8-HQS + 50 g l^{-1} sucrose (Table 2).

The results in Table 2 show that all the STS treatments gave a higher fresh weight % than the control, while the fresh weight % was reduced somewhat when sucrose was added, at all concentrations of STS. The highest fresh weight % of 98.9% was obtained with 0.2 mM STS without sucrose, compared with 88.2% in the control.

Treatment with 1-MCP led to an increase in the fresh weight %, the highest value being obtained after treatment with 1-MCP at 0.5 g m^{-3} for 6 h (Table 2).

Chlorophyll content

The use of different chemical treatments led to a significant delay in chlorophyll loss (chl *a* and chl *b*). All the chemical treatments minimized the chlorophyll loss compared with the untreated control. The best treatment in this respect was 0.4 mM STS + 50 g l^{-1} sucrose. At the end of the experiment the chlorophyll content in the leaves was higher after this treatment than in the control or in the other treatments (Table 3).

Table 1
Effect of 8-HQS, STS and 1-MCP on the vase life of *Dianthus caryophyllus* L. cv. Asso

Treatments	Vase life (Days)
Control	8.3a
200 ppm HQS+50 g l^{-1} sucrose	18.3ef
200 ppm HQS	11.0b
400 ppm HQS+50 g l^{-1} sucrose	19.3f
400 ppm HQS	11b
0.2 mM STS+50 g l^{-1} sucrose	22.7gh
0.2 mM STS	21.7g
0.4 mM STS+50 g l^{-1} sucrose	23.7h
0.4 mM STS	23.3gh
1-MCP 0.3 g m^{-3} 6 h	15.3c
1-MCP 0.5 g m^{-3} 6 h	17.3de
1-MCP 0.7 g m^{-3} 6 h	16.3cd

Means followed by different letters differ significantly from each other according to Duncan's multiple range test at $P = 0.05$.

Table 2
Effect of 8-HQS, STS and 1-MCP on the % fresh weight of *Dianthus caryophyllus* L. cv. Asso

Treatments	Initial fresh weight (g)	Fresh weight at the end of the experiment (g)	Fresh weight as a % of initial weight*
Control	16.9	14.9	88.2ab
200 ppm HQS+50 g l^{-1} sucrose	17.7	19.7	111.3de
200 ppm HQS	18.9	18.7	99bcd
400 ppm HQS+50 g l^{-1} sucrose	19.8	23.3	117.7e
400 ppm HQS	18.7	20.0	107cde
0.2 mM STS+50 g l^{-1} sucrose	18.2	17.4	95.6a
0.2 mM STS	18.5	18.3	98.9bcd
0.4 mM STS+50 g l^{-1} sucrose	17.4	16.7	96bcd
0.4 mM STS	17.6	17.0	96.6bcd
1-MCP 0.3 g m^{-3} 6 h	19.7	18.3	92.9abc
1-MCP 0.5 g m^{-3} 6 h	20.6	19.8	96.1bcd
1-MCP 0.7 g m^{-3} 6 h	21.0	18.8	89.5ab

Means followed by different letters differ significantly from each other according to Duncan's multiple range test at $P = 0.05$. *The statistical analysis is valid only for the last column (fresh weight as a % of initial weight).

Discussion

The results show the importance of 8-HQS in increasing the vase life of cut carnations. These results may be due to the role of 8-HQS as an anti-microbial agent, which could thus reduce stem plugging. The results could also be explained through the maintenance of leaf turgidity, and by the fact that fresh weight and chlorophyll losses were kept to a minimum.

Table 3
Effect of the best treatment of each chemical on the chlorophyll content (mg g^{-1} fresh weight) of the leaves of cut carnations

Treatments	Day 3		End of experiment	
	chl *a*	chl *b*	chl *a*	chl *b*
Control	0.91a	0.53a	0.63a	0.42a
8-HQS 400 ppm+50 g l^{-1} sucrose	1.46b	0.68b	1.12b	0.53b
STS 0.4 mM+50 g l^{-1} sucrose	1.63c	0.69b	1.33c	0.55bc
1-MCP 0.5 g m^{-3} 6 h.	1.63c	0.72c	1.23bc	0.57c

Means followed by different letters differ significantly from each other according to Duncan's multiple range test at $P = 0.05$. Results were analysed separately for each sampling day, so the statistical analysis is only valid within a column.

These results are in agreement with the findings of Hussein (1994) on cut flowers of chrysanthemum and calendula. Knee (2000) also found that the blockage of xylem elements by microorganisms was prevented by using HQC and the vase life of cut carnations was increased. This could be attributed to the role of 8-HQS in increasing the level of absorbance, as the result of which, the vase life was increased (Hassan et al., 2003).

STS is a very potent inhibitor of ethylene action in plant tissues. It also provides antimicrobial activity inside the plant tissues, and is thus beneficial for ethylene-sensitive flowers such as carnation (Nowak and Rudnicki, 1990). In addition, in the STS treatment the percentage weight loss and chlorophyll degradation was minimized and consequently the vase life was extended. These results are in harmony with the results obtained by Menguc and Usta (1994) and Celikel et al. (1995) on cut carnations.

Concerning the role of sucrose in combination with 8-HQS or STS, it is well known that the sugar supply increases the longevity of many cut flowers. While sucrose can act as a source of nutrition for tissues approaching carbohydrate starvation, it may also act as an osmotically active molecule, thereby having a role in flower opening and subsequent water relations (Kuiper et al., 1995). Similar findings were obtained by Erin et al. (2002), who found that vase solutions containing sugar improved the vase life of many cut flower crops.

The extension of the vase life of cut carnations using 1-MCP could be attributed to the role of 1-MCP as an inhibitor of ethylene biosynthesis and ethylene binding, consequently preventing the undesirable post-harvest effects of ethylene, as reported by Serek et al. (1995). The fact that the leaves were kept in good condition by lowering the percentage weight loss and retarding chlorophyll degradation may also have led to an increase in vase life. In this connection, Celikel and Reid (2002) reported that, even in the absence of exogenous ethylene, the life of the flowers was significantly increased by inhibiting ethylene action using pretreatment with 1-MCP. Similar results were obtained by

Sisler and Serek (2001), who reported that treatment with 1-MCP protected carnation flowers against ethylene for several days and consequently the vase life was extended. It was also reported that treatment with 1-MCP at 0.5 g m^{-3} for 6 h increased the vase life and minimized the % loss of initial weight in cut carnations (Hassan and Gerzson, 2002).

Conclusions

All the chemicals used improved the post-harvest quality of cut carnations and it could be concluded from the results that 1-MCP is an effective blocker of ethylene perception in cut carnations. Furthermore, its non-toxic character makes the material an excellent replacement for the environmentally unsafe silver ion. It should, however, be noted that these results are valid for an ethylene-free atmosphere and would be different if the flowers were exposed to ethylene. Consequently, the next step in the experiments will be to apply the treatment in the presence of ambient (external) ethylene.

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CONCENTRATION AND UPTAKE OF SECONDARY NUTRIENTS (Ca, Mg, S) IN RICE AS INFLUENCED BY DURATION OF VARIETY AND NITROGEN FERTILIZATION

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A field experiment was conducted for 2 years at the Indian Agricultural Research Institute, New Delhi to study the effects of duration of variety and nitrogen fertilization on the Ca, Mg and S concentration and uptake in rice. In general, the concentrations of Ca, Mg and S were not significantly influenced by the duration of the rice variety. N fertilization had a tendency to increase the concentration of Ca, Mg and S at 45 days after transplanting as well as at harvest; however, the difference was significant only in the case of the Ca concentration in the grain and only when the N level was raised from 60 to 120 kg N/ha. The duration of the rice variety had a significant effect on the Ca, Mg and S uptake by the straw and grain at harvest, which was higher in the medium duration variety Pusa Basmati-1 than in Pusa Jaldi Dhan-1, mainly due to the higher yields obtained with the former cultivar. Nitrogen application significantly increased the Ca, Mg and S uptake at harvest mainly due to its increasing effects on the grain and straw yields of rice. The average uptake of Ca, Mg and S was 12.6, 13.6 and 3.5 kg per metric ton of grain, respectively.

Key words: rice, nitrogen, short/medium duration, calcium, magnesium, sulphur, secondary nutrients

Introduction

Rice is the staple food of millions of poor people in Asia, where 90% of the world's rice is grown and eaten (IRRI, 1989). However, the population in rice-consuming countries is increasing at a much faster rate than in the rest of the world, and Pinstrup-Anderson (1994) opined that the demand for rice will soon exceed the production. Continued efforts are therefore essential to increase the rice production in the world. Before the introduction of medium high-yielding varieties the net removal of plant nutrients was small and even poor soils had the capacity to supply sufficient amounts to sustain the low yield levels of traditional cultivars. The modern high-yielding varieties yield twice or three times the yields obtained with the traditional cultivars and call for adequate fertilization with major plant nutrients. The good responses of high-yielding varieties of rice to NPK application have been reported all over Asia (Tanaka et al., 1964; De Datta et al., 1988; Tandon, 1987; 1989; Lakshmanan and Prasad, 1998; Dobermann et al., 1998) and China now tops the world in fertilizer consumption, which was 34.2 million tones ($N+P_2O_5+K_2O$) in 2000–01, while India was third in the world with a consumption of 16.7 million tonnes (FAI, 2002). Thus, the consumption of major primary plant nutrients has considerably

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increased in the rice-growing countries of Asia. As regards secondary plant nutrients, only S has received attention in recent years and its deficiency is reported to be widespread (Fan and Messick, 1997; Sarkar, 2000; Katyal and Rattan, 2003). The main factors responsible for this are the high yields of modern rice cultivars, resulting in the increased removal of S from soils and the replacement of S-containing fertilizers such as ordinary superphosphate and ammonium sulphate by the non S-containing fertilizers diammonium phosphate and urea. Due to the abundance of Ca and Mg in the soils their deficiencies in rice have not been reported (Biswas et al., 1985). Most of the information available on Ca application to rice is from studies on soil amendments: lime on acid soils (Sarkar and Singh, 2003) and gypsum on salt-affected soils (Swarup, 2003). Little information is available on the Mg nutrition of rice. The demand for short-duration rice varieties is increasing in order to increase the intensity of cropping in rice-based cropping systems in Asia to produce more grain per hectare. No information is available on the comparative nutrient uptake by short (70–75 days) and medium duration (130–135 days) varieties. The present study was therefore carried out to study the effect of the duration of the cultivars and of nitrogen fertilization on the Ca, Mg and S concentration and uptake in rice.

Materials and methods

The field experiments were conducted during the wet season (July–November) of 1990 and 1991, the main rice growing season of northwestern India. A split plot design with varieties (Pusa Jaldi Dhan-1 and Pusa Basmati-1) in the main plots and nitrogen levels (0, 60, 120 kg N ha^{-1}) in the sub-plots was adopted. There were three replications. The soil of the experimental field was a sandy clay loam alluvium of pH 8.0 with 0.75% organic C and medium quantities of available P and K. The exchangeable Ca, Mg (Hendershot and Duquette, 1986) and available S (Palaskar et al., 1981) was 3.8, 5.0 and 16.5 mg/kg soil.

Pusa Jaldi Dhan-1, developed at the Indian Agricultural Research Institute, New Delhi from the cross Gora M/MW-10M/N-22M (M is an induced mutant; N-22 refers to Nagina 22), is a medium tall variety with aggressive early growth and a crop duration of 70–75 days. It is specially suitable as a catch crop in intensive multiple cropping systems or for post-flood sowing on flood-prone areas (*diara* lands).

Pusa Basmati-1, developed at the Indian Agricultural Research Institute, New Delhi, is a cross between Pusa 167 and Karnal Local Basmati. It is a semi-dwarf variety of 135–140 days' duration and has long slender aromatic grains. It is suited for irrigated rice culture in northwestern India.

Methodology

The experimental field was disk-ploughed twice, puddled with a country plough in standing water and finally levelled. Before sowing, 22 kg ha^{-1} each of P (as ordinary superphosphate) and K (as muriate of potash) and 5 kg ha^{-1} of $ZnSO_4$ were applied as basal fertilizer. Two to three 25-day-old seedlings were transplanted per hill (at 20 cm × 10 cm spacing). Half the N dose was applied 10 days after transplanting (DAT) when the plants were established. The rest of the N was applied 20 days after the application of the first dose. The crop was irrigated as and when necessary (8 irrigations in 1999 and 10 irrigations in 1991).

Samples of plants at 45 days after transplanting (active vegetative growth stage) and of grain and straw at harvest were taken from each plot, dried at 60±5°C and finally ground on a Wiley mill. The samples were digested in a diacid mixture consisting of 9 parts nitric acid and 4 parts perchloric acid (Prasad, 1998) and analysed for total Ca and Mg (Ghosh et al., 1983) and S (Palaskar et al., 1981). The uptake of Ca, Mg and S by rice at the 45 DAT stage and in the grain and straw at harvest was computed by multiplying the concentration of these nutrients by the respective dry weights.

Results and discussion

Calcium

The data on Ca concentration and uptake in rice plants 45 days after transplanting (DAT) and the grain and straw at harvest are presented in Table 1. The Ca concentration in rice plants and straw at harvest varied from 3.4 to 4.7 g/kg for the two varieties, averaging about 4 g/kg. These values are in accord with those reported by Loneregan and Snowball (1969) for monocots. Pusa Basmati-1 had a significantly higher Ca concentration than Pusa Jaldi-1 at 45 DAT in 1990. The Ca concentration in the grain of the two rice varieties did not differ significantly and was much lower compared to that in the straw. This was expected, because Ca is not a mobile nutrient in plants (Prasad and Power, 1997). The Ca concentration in rice was not significantly affected by N fertilization at 45 DAT, but at harvest the Ca concentration in the straw was significantly higher in plots receiving N and a significant difference was recorded between 60 and 120 kg N/ha in 1991. The Ca concentration in rice grains also increased with N fertilization and the increase at 120 kg N/ha over 60 kg N/ha was significant in both years of the study. Thus, N fertilization helped the translocation of Ca from plant tissues to the grain. This was not unexpected, because even in rice, part of the N is taken up as nitrates, and Ca, as the most abundant cation to be associated with it in the soil solution, is thus absorbed by the plants.

The Ca uptake by the plants at 45 DAT and in the straw and grain at harvest was significantly higher in the variety Pusa Basmati-1 than for Pusa Jaldi-1 and this difference was significant at harvest. This was mainly due to the higher grain and straw yields obtained with Pusa Basmati-1 (Lakshmanan and Prasad, 1998; see also Table 4). Nitrogen fertilization increased the Ca uptake by rice at 45 DAT as well as at harvest, both due to the increase in Ca concentration, as discussed above, and due to the increase in biomass at 45 DAT and straw and grain yield at harvest. The increase in Ca uptake in the 120 kg N/ha treatment was significantly more than at 60 kg N/ha, which in turn was superior to the control. The total (grain + straw) Ca uptake by rice at harvest ranged from 16.6 to 57.2 kg/ha (Table 4) with an average of 32.8 kg Ca/ha. In terms of kg Ca per metric tonne (t) of rice grain, the values ranged from 9.8 to 15.1 (Table 4) with a mean of 12.6 kg Ca/t grain.

Table 1
Ca concentration and uptake in rice as influenced by duration of variety and N fertilization

Factor	Ca concentration (g/kg)						Ca uptake (kg/ha)					
	45 DAT		Straw		Grain		45 DAT		Straw		Grain	
	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991
Variety												
Pusa Jaldi	3.4	4.2	4.0	4.7	0.8	0.9	10.1	16.1	15.7	16.8	0.9	1.2
Pusa Basmati-1	4.2	4.0	4.2	4.0	0.9	0.9	12.2	14.4	53.7	42.6	3.5	4.5
$LSD_{5\%}$	0.1	NS	NA	NA	NA	NS	NS	NS	9.5	17.9	0.6	2.2
Kg N/ha												
0	2.8	3.7	3.7	3.4	0.7	0.7	6.4	5.6	28.1	17.3	1.5	1.3
60	3.0	4.1	4.0	3.9	0.8	0.8	9.8	12.9	32.3	25.4	2.0	2.3
120	3.4	4.2	4.5	4.2	0.9	1.1	13.0	18.9	40.0	34.9	2.5	3.5
$LSD_{5\%}$												
0 vs 60	NS	NS	NS	NS	NS	NS	3.3	5.3	NS	4.5	0.4	0.9
60 vs 120	NS	NS	NS	0.3	0.1	0.2	2.4	3.8	5.0	3.2	0.3	0.7

NS – Not significant; NA – Not analysed

Magnesium

The data on the Mg concentration and uptake in rice plants at 45 DAT and in the grain and straw at harvest are presented in Table 2. The Mg concentration in rice plants at 45 DAT and in the straw at harvest ranged from 2.9 to 4.6 g/kg, while that in the grain ranged from 1.2 to 1.5 g/kg. Neither the duration of the rice variety nor the N fertilization significantly influenced the Mg concentration in the rice plants at 45 DAT or in the grain and straw at harvest. The Mg concentration in the grain was greater than that of Ca (Table 1), showing that Mg is a mobile nutrient in plants (Prasad and Power, 1997).

The Mg uptake in the two rice varieties was not significantly different at 45 DAT. However, at harvest the N uptake in both the grain and straw was significantly greater in the medium duration rice variety Pusa Basmati-1 than in the short duration variety Pusa Jaldi-1, mainly due to the increase in yield. N fertilization increased the Mg uptake in rice plants at 45 DAT and the difference between 120 kg N/ha and 60 kg N/ha was significant in both the years of study. The Mg uptake by the rice straw and grain at harvest increased as the level of N increased; however, the difference between the levels was only significant in the case of straw in 1991. The total (grain + straw) Mg uptake of rice at harvest ranged from 16.9 to 63.1 kg/ha with an average of 34.2 kg/ha. In terms of kg Mg per metric tonne of rice, the values ranged from 9.0 to 16.7 (Table 4) with a mean value of 13.6 kg Mg/t rice grain.

Table 2
Mg concentration and uptake in rice as influenced by duration of variety and N fertilization

Factor	Mg concentration (g/kg)						Mg uptake (kg/ha)					
	45 DAT		Straw		Grain		45 DAT		Straw		Grain	
	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991
Variety												
Pusa Jaldi	4.3	3.9	4.3	4.4	1.5	1.3	15.7	14.9	16.9	15.4	1.5	1.5
Pusa Basmati-1	4.3	3.8	4.6	2.9	1.3	1.4	14.1	13.4	57.8	37.7	5.3	7.1
$LSD_{5\%}$	NS	NS	NA	NA	NS	NS	NS	NS	3.1	10.6	0.4	2.2
Kg N/ha												
0	4.2	3.7	4.6	3.4	1.3	1.2	10.0	5.4	33.8	17.3	2.7	2.2
60	4.3	3.8	4.5	3.5	1.3	1.3	13.7	11.9	36.2	23.1	3.3	3.8
120	4.3	3.9	4.6	3.6	1.4	1.5	16.7	17.5	38.8	30.6	3.7	5.0
$LSD_{5\%}$												
0 vs 60	NS	NS	NS	NS	NS	NS	2.9	4.9	NS	4.5	NS	NS
60 vs 120	NS	NS	NS	NS	NS	NS	2.1	3.5	NS	3.2	NS	NS

NS – Not significant; NA – Not analysed

Sulphur

The data on the S concentration and uptake in rice plants at 45 DAT and in the grain and straw at harvest are presented in Table 3. The S concentration in rice plants ranged from 1.5 to 2.6 g/kg and that in the straw from 0.4 to 1.5 g/kg and was not significantly affected by the duration of the variety or by N fertilization. The S concentration in the rice grain ranged from 0.7 to 1.3 g/kg and was significantly higher in the short-duration variety Pusa Jaldi-1 than in Pusa Basmati-1. This could be due to the higher grain yield of Pusa Basmati-1.

The S uptake by rice plants at 45 DAT was not significantly affected by the duration of the rice variety but increased significantly with each successive level of N, the highest S uptake being recorded at 120 kg N/ha. The sulphur uptake in the rice straw at harvest was significantly greater in Pusa Basmati-1 than Pusa Jaldi-1 in 1990 only, while a significant increase due to N fertilization was recorded in 1991 only, when the S uptake was the highest at 120 kg N/ha. The sulphur uptake in the rice grain was greater in Pusa Basmati-1 than in Pusa Jaldi-1 in both the years despite a lower S concentration in the grain, mainly due to the much higher yield obtained in Pusa Basmati-1 (Table 4). The sulphur uptake by the rice grain also increased with N fertilization, and at 120 kg N/ha gave a higher S uptake than at 60 kg N/ha in 1991.

The total (grain + straw) uptake of S by rice at harvest ranged from 6.8 to 14.4 kg/ha (Table 4) with an average of 8.6 kg S/ha. Sarkar et al. (2000) reported the removal of 7.8 to 22.6 kg S/ha by rice in north Bihar, while Savithri et al. (2000) reported values of 22.1 to 34.7 kg S/ha removal by rice in Tamil Nadu in India. In terms of kg S per metric tonne of rice grain the values ranged from 2.0 to 6.3 (Table 4) with a mean value of 3.5 kg S/t rice grain.

Table 3
S concentration and uptake in rice as influenced by duration of variety and N fertilization

Factor	S concentration (g/kg)						S uptake (kg/ha)					
	45 DAT		Straw		Grain		45 DAT		Straw		Grain	
	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991
Variety												
Pusa Jaldi	2.5	2.5	1.4	1.5	1.3	1.2	9.2	9.3	5.3	5.4	1.4	1.4
Pusa Basmati-1	2.3	1.5	0.8	0.6	0.9	0.7	7.6	5.5	10.7	6.8	3.7	3.6
$LSD_{5\%}$	NS	NS	NA	NA	0.2	0.4	NS	NS	1.9	NS	0.8	1.3
Kg N/ha												
0	2.0	1.8	0.7	0.4	0.9	0.8	4.9	2.8	6.0	2.7	1.7	1.2
60	2.2	1.9	0.8	0.5	1.1	0.9	7.1	6.0	7.6	5.0	2.6	2.1
120	2.6	2.1	0.9	0.7	1.2	1.1	9.9	9.2	8.7	7.5	2.7	3.0
$LSD_{5\%}$												
0 vs 60	NS	NS	NS	NS	NS	NS	1.5	2.9	NS	2.1	0.7	NS
60 vs 120	0.2	NS	NS	NS	NS	NS	0.5	2.0	NS	1.5	NS	0.8

NS – Not significant; NA – Not analysed

Table 4
Total Ca, Mg and S uptake in rice at harvest

Factor	Grain		Ca				Mg				S			
	t/ha		kg/ha		kg/t grain		kg/ha		kg/t grain		kg/ha		kg/t grain	
	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991
Variety														
Pusa Jaldi	1.1	1.1	16.6	18.0	15.1	16.4	18.4	16.9	16.7	15.4	6.9	6.8	6.3	6.2
Pusa Basmati-1	4.1	5.0	57.2	47.1	13.9	9.4	63.1	44.8	15.4	9.0	14.4	10.4	3.5	2.1
Kg N/ha														
0	2.2	1.9	29.6	18.6	13.4	9.8	36.5	19.5	16.6	10.3	7.7	3.9	3.5	2.0
60	2.6	2.8	34.3	28.7	11.0	10.3	38.3	26.9	14.7	9.6	10.2	7.1	3.9	2.5
120	2.6	3.4	40.0	38.4	15.4	11.3	42.5	35.6	16.3	10.5	11.4	10.5	4.4	3.1

t = metric tonne = 1000 kg

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EFFECT OF WATER QUALITY ON GRAIN YIELD AND NUTRIENT UPTAKE OF RICE (*Oryza sativa* L.)

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The use of poor quality water for agriculture is now receiving major attention especially in arid and semi-arid regions. This experiment was carried out to evaluate the effects of different irrigation water qualities on the grain yield and nutrient uptake of rice and on the heavy metal concentration in the grains. Six water treatments were applied at intervals of three days, involving either fresh water (FW), drainage water (DW), mixed water (MW), fresh water followed by drainage water (1FW + 1DW), two applications of fresh water followed by one of drainage water (2FW + 1DW) or one application of fresh water followed by two of drainage water (1FW + 2DW). The rice grain yield and the uptake of nitrogen (N), phosphorus (P) and potassium (K) were determined. The grains were also analysed for the concentration of nickel (Ni), cadmium (Cd) and lead (Pb). The results showed that the grain yield, the uptake of N, P and K in the plant biomass and the concentration of heavy metals in the grains were significantly affected by the water quality. The rice grain yield exhibited a close correlation with the water quality. The highest grain yield was obtained in the FW treatment and the lowest yield in the DW treatment. The uptake of N, P and K was detrimentally affected by poor quality water. However, the uptake trend for these elements was similar across all the irrigation treatments. The concentrations of heavy metal in the grains were significantly higher in plots irrigated with poor quality water. Among the treatments the cumulative concentrations of heavy metal were in the order of: DW >1FW + 2DW > MW > 1FW + 1DW > 2FW + 1DW > FW. This study showed that there is a potential risk of heavy metal contamination in rice crops treated with poor quality water. The lower grain yield after irrigation with poor quality water could be due to the disturbed mineral nutrition or to relatively higher salt toxicity.

Key words: grain yield, heavy metals, nutrient uptake, poor quality water, rice

Introduction

There is a shortage of water resources for agriculture in the arid regions of the world. Therefore, it is necessary to utilize water with marginal quality to irrigate crops. The use of saline drainage waters in such environments shows promise for growing agricultural crops (Rhoades, 1987). Rhoades et al. (1992) reported the use of domestic drainage or waste water at the rate of 4.7 billion cubic metres (bcm) per annum for irrigation purposes and predicted that this would increase to 7 bcm per annum by the year 2000. Abou-Hussien and El-

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Koumey (1997) also reported an increase in the re-use of water for agriculture with growing population, urban and industrial development. The environmental effects of agricultural irrigation and drainage are widespread and significant. The quality of irrigation water varies and depends on the type and quantity of salts, heavy metals, nitrate, oil, etc. In arid and semi-arid regions, where soil fertility is low, the concept of irrigation with poor water quality is gaining priority. Nutritional deficiencies and elemental toxicities in crops are related to a number of soil and plant factors. Plants respond differently to saline waters and the response of the crop depends on the type and frequency of irrigation (Mass, 1990). The suppression of plant growth under saline conditions is related to either a reduction in osmotic pressure or a specific ion effect (Abd Allah, 1995). The major constraint in the utilization of poor quality water is the perception of contamination of the environment by heavy metals and/or pathogens. The risk associated with the accumulation of trace metals includes the contamination of the terrestrial food chain and phytotoxicity. The potential hazard of heavy metals with the application of such waters may also arise in paddy fields.

Several studies have been made on the effects of waste water on the soil-plant system but few reports have been published on the influence of waste water on the uptake of major nutrients and trace metals, especially under paddy soil conditions. Therefore, it is necessary to develop a preliminary understanding on the safe utilization of waste water in paddy soils. This study aimed to evaluate the effect of the quality of irrigation water on the rice grain yield, the uptake of major nutrients (N, P and K) and the concentration of heavy metals (Cd, Ni and Pb) in grains under paddy soil conditions.

Materials and methods

The research area was located at the end of the River Nile in the north of Egypt, where there is a shortage of fresh water and the soils have recently been reclaimed. The fields contain two canal systems, for irrigation and drainage purposes. When fresh water is scarce, farmers also use drainage water for irrigation. Therefore, this study was designed to evaluate the response of rice to the combined effects of drainage water and fresh water. The experiment was conducted on the farm of the Rice Research and Training Center, Sakha Kafr El-Shiekh, Egypt during the years 2000 and 2001. The soil was analysed for physico-chemical properties. The samples were air-dried and screened to pass a 2 mm sieve. Soil texture was determined by the pipette method. Organic matter was determined by the wet combustion method (Jackson, 1965). The electrical conductivity (EC) and pH of the soil were measured in a 1:5 soil-water solution using pH and EC meters consecutively. Soluble phosphorus was determined colorimetrically by the ascorbic acid method (Watanabe and Olsen, 1965). The contents of soluble Ca, Mg, Na and K were determined using an atomic absorption spectrophotometer (AAS) and the soluble anions Cl^-, $SO4^{2-}$, CO_3^{2-} and HCO_3^- were determined according to the methods described by Richards (1954). The concentrations of heavy metals were determined using an AAS in a DTPA extract (100 ml 0.05M DTPA per 10 g of soil) shaken for 2 hours at room temperature and then filtered (Lindsay and Norvell, 1978). The soil properties are given in Table 1.

Table 1
Physico-chemical properties of the soil used in the experiment

Soil analysis	Unit	Amount
Sand	%	11.59
Silt	%	32.93
Clay	%	55.48
Texture class		Clay
EC	dSm^{-1}	1.20
pH		8.16
Organic matter	%	1.50
Total N	$mg\ kg^{-1}$	45.0
Soluble P	$mg\ kg^{-1}$	20.0
Soluble cations		
Na	$meq\ kg^{-1}$	2.33
K		1.17
Ca		3.00
Mg		2.22
Soluble anions	$meq\ kg^{-1}$	
HCO_3		2.98
Cl		8.50
SO_4		4.76
Available heavy metal	$mg\ kg^{-1}$	
Cd		0.01
Ni		0.18
Pb		0.14
Zn		0.70

The influence of different qualities of water on the rice crop (cv. Sakha 101) was investigated. The crop was irrigated at intervals of three days with the following treatments: fresh water (FW), drainage water (DW), mixed water, consisting of equal ratio of fresh and drainage water (MW), fresh water followed by drainage water (1FW + 1DW), fresh water on two occasions followed by drainage water (2FW + 1DW), one application of fresh water and two of drainage water (1FW + 2DW). The electrical conductivity (EC_w) and pH of the irrigation water were measured. The EC_w of DW was obtained as 2.7 dSm^{-1}. Rhoades et al. (1992) classified water with EC (dSm^{-1}) in the 2.0–10.0 range as moderately saline. The heavy metal content in the water samples was analysed using AAS. The chemical characteristics of the water samples (Table 2) were determined according to the procedures of Klute (1986). The sodium adsorption ratio (SAR) of the irrigation water was calculated using the following equation:

$$SAR = \frac{Na}{\sqrt{\frac{Ca + Mg}{2}}}$$

where Na, Ca and Mg are expressed in milliequivalents per litre.

The fertilizers were applied according to the conventional application rates adopted at the research farm. Phosphorus was applied at the rate of 36 kg P ha^{-1} as calcium monophosphate in dry soil. Zinc was added as $ZnSO_4$ at the rate of 24 kg Zn ha^{-1}. Nitrogen was applied as urea at the rate of 150 kg N ha^{-1} in splits, 2/3 being incorporated before irrigation and 1/3 at the panicle initiation stage, 30 days after transplanting. The treatments were arranged in a randomized complete block design with four replications. The experiment was repeated in the year 2001. The

data of all parameters for 2000 and 2001 were calculated as the means of the two years. Each plot consisted of 50 m^2. The seeds were sown on 1st May. The 25-day-old screened seedlings were transplanted with three seedlings per hill at a spacing of 20 × 20 cm. The heads emerged at the end of August and the crop was harvested in the second half of October. The rice grain yield was recorded, as were the uptake of macroelements in the shoot after harvest and the concentration of heavy metals in the rice grains.

Five hills of matured rice plants with an even number of panicles in each plot were sampled. The plant samples were dried in an oven at 105°C for two hours and then at 75°C for 3 days. The plants were threshed and the grain yield was calculated on the basis of 5 m^2. The yield was calibrated for 14% moisture content as described by Wang et al. (1997). The shoot samples were ground to a powder and thereafter digested in a mixture of sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) at 240–260°C for 5 hours. The nitrogen concentration was determined with the modified micro-Kjeldahl distillation method (Concon and Soltess, 1973). Phosphorus was determined colorimetrically with a spectrophotometer. Potassium was determined using a flame emission spectrophotometer. The uptake of N, P and K in the rice straw was determined by multiplying the concentration of these elements by the straw dry weight. The rice grains were air-dried, hulled and screened. For each treatment a 1 g grain sample was placed in a beaker and digested with a mixture of concentrated HNO_3 and $HClO_4$ (1:1) on a hotplate. The concentrations of Ni, Cd and Pb in the grains were determined using AAS. The irrigation treatments were compared for nutrient uptake, grain yield and heavy metal contents of the grains using LSD at the 0.05 level of significance.

Results and discussion

Grain yield

Irrigation with poor water quality resulted in a lower rice grain yield than fresh canal water (Table 3). The grain yield was highest in FW treated plots and lowest in DW. The grain yield decreased by 8% in MW, 14.6% in 1FW + 2DW and 16.0% in DW as compared to the FW treatment. However, the yield did not differ significantly between the FW, 2 FW + 1DW and 1FW + 1DW treatments. The reduced yield observed in the low quality water treatments might be due to the presence of higher contents of salts and/or heavy metals in the drainage water, which impaired the yield potential of the plants. It has been reported by Zurayk et al. (2001) that salts in the drainage water reduced the biomass yield of crops. Ragheb et al. (1993) reported an increased concentration of salts and reduced total dry matter and grain yield in wheat due to the application of poor quality water.

Nutrient uptake

The uptake of N, P and K was significantly affected by the quality of the irrigation water (Table 3). The results indicated that the N uptake was significantly reduced by poor water quality. Among the irrigation treatments the N uptake differed in the order of 2FW + 1DW > 1FW + 1DW > FW > MW > 1FW + 2DW > DW (Table 3). The decreased N uptake could have been due to the nutritional imbalance under higher salt stress. Pessarakli and Tucker (1985) reported that an excess of salt in the soil or nutrient solution caused a decrease in the total N uptake by plants.

Table 2
Some chemical characteristics of the irrigation water used

Property	Water quality*		
	FW	DW	MW
pH	7.80	8.10	8.14
EC_w (dSm^{-1})	0.79	2.66	1.93
Cations (meq l^{-1})			
Na	3.04	22.88	15.20
K	0.22	0.32	0.26
Ca	2.20	3.00	2.60
Mg	1.20	2.80	2.40
Anions (meq l^{-1})			
HCO_3	3.80	6.80	5.00
Cl	1.60	2.40	2.20
SO_4	1.26	19.80	13.26
SAR	1.65	9.49	6.79
Heavy metals (mg l^{-1})			
Cd	0.03	0.09	0.06
Ni	0.19	0.91	0.68
Pb	0.26	0.33	0.29

* In this and the subsequent tables FW, DW and MW denote fresh water, drainage water and mixed water.

Table 3
Effect of the quality of irrigation water on the grain yield (t ha^{-1}) and the N, P and K uptake (g m^{-2}) in rice straw

Water quality treatments	Grain yield	N	P	K
FW	10.56	28.0	15.0	39.1
2FW + 1DW	10.82	28.2	15.0	39.0
1FW + 1DW	10.63	28.1	14.5	31.5
MW	9.71	27.6	13.2	29.0
1FW + 2DW	9.02	26.7	12.8	28.0
DW	8.87	22.1	11.0	27.5
$LSD_{0.05}$	0.45	0.32	0.55	1.87

The uptake of phosphorus (P) also decreased when low quality water was applied. The P uptake in 2FW + 1DW and 1FW +1DW was not significantly different from FW, whereas the P uptake decreased significantly in 1FW + 2DW, MW and DW as compared to FW. The low uptake in low quality water treatments could have been due to ionic strength effects that reduce the activity of phosphate or to the low solubility of P caused by the presence of salt in DW. Sharpley et al. (1992) also reported a decreased P uptake in crops with increased salts levels.

Irrigation with poor quality water significantly decreased the K uptake in rice, as in the case of P and N. The trend of K uptake was similar to that of N and P for all types of water application. This similar trend in the uptake of essential elements indicated that their uptake is highly dependent on each other within the plant. Ahmed et al. (1987) reported that K is one of the most

important elements affecting the N metabolism of the rice plant. El-Gayer et al. (1986) reported that reductions in K concentration could relate to the higher osmotic pressure and salinity level of the irrigation water. The absorption of K by rice plants differed widely according to the composition and type of irrigation water applied (Atwa, 1999). Classen and Wilcox (1974) reported that the uptake of K by plants was reduced due to salt stress. Okusanya and Ungar (1984) reported that the K content in plant tissues was reduced as the salinity increased.

The growth and nutrient concentrations in plants usually determine their performance in any environment. The uptake of major elements (N, P and K) in the rice straw was determined by multiplying their concentrations by straw dry weight. The present experiment demonstrated that crop yield and nutrient concentrations were significantly influenced by the source of water. As the uptake of nutrients is related to the straw biomass, the lower uptake of these elements could have resulted from the relatively lower biomass yield due to the poor quality of the irrigation water.

Heavy metal concentrations in grains

The application of waste water resulted in a greater concentration of heavy metals (Ni, Cd and Pb) in the grains. Rice plants grown in the MW, 1FW + 2DW and DW irrigation treatments absorbed higher amounts of Ni, Cd and Pb in the grains as compared to the other irrigation treatments. Among the heavy metals the concentrations decreased in the order Ni > Pb > Cd across all types of water treatments. In the DW treatment the average contents of Ni, Cd and Pb (mg kg^{-1}) were found to be 0.09, 1.46 and 0.58, respectively (Table 4). The higher concentration of heavy metals in the grains could be due to the presence of higher amounts in the DW. The concentrations of these heavy metals (mg kg^{-1}) exceeded the limits given by FAO (1983), i.e. 0.08 for Cd and 2.80 for Pb. The concentrations reported by Lin (1991) for Cd, Ni and Pb (mg kg^{-1}) rice grains were 0.07, 0.43 and 0.54, respectively. Trace elements such as Se, Cd, Cr and Ni may occur in appreciable concentrations in drainage water, as the result of anthropic activity or geo-characteristics (Deverel and Fujii, 1990). Han et al. (2000) reported that organic wastes which are included in waste water carry variable toxic elements. Tsaplina (1993) reported that heavy metals are aggressive environmental pollutants. The higher uptake of metals by plants may create greater stress and disturb plant metabolism.

Table 4

Concentrations (mg kg^{-1}) of Cd, Ni and Pb in rice grains as affected by the quality of irrigation water

Water quality treatments	Cd	Ni	Pb	Total
FW	0.02	1.01	0.20	1.23
2FW + 1DW	0.02	1.13	0.21	1.36
1FW + 1DW	0.04	1.19	0.26	1.49
MW	0.03	1.48	0.50	2.01
1FW + 2DW	0.06	1.48	0.60	2.14
DW	0.09	1.46	0.58	2.13
$LSD_{0.05}$	0.02	0.18	0.07	0.27

This study indicates that water quality has detrimental effects on the rice grain yield and nutrient uptake. Poor water quality interfered with the uptake of N, P and K, whereas the concentration of heavy metals was enhanced in the rice grains. There is a risk of contamination associated with the continuous application of waste water. Therefore, irrigation with waste water must be carefully rationalized, especially for food crops. The hazards of heavy metals can possibly be reduced through the use of drainage water in combination with fresh water. Sustained efforts are needed to evaluate the safe use of waste water in the soil-plant–system.

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INTERACTION OF SALINE WATER AND NITROGEN ON THE PARTITIONING AND STATISTICAL CORRELATION OF MINERAL ELEMENTS IN MAIZE PLANTS

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Saline irrigation water has a tremendous impact on the yield potential of crops. The distribution of mineral elements and their ratios in maize plant organs in response to saline water and nitrogen (N) nutrition was studied in a pot experiment for six weeks. The plants were separated into leaf, stalk and root and analysed for calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl) contents. The partitioning and ratios of mineral nutrients in plants were significantly affected by water salinity and nitrogen level. In saline water the roots contained the highest Na content; Ca and Mg were higher in the leaf, whereas K and Cl were highest in the stalk. In non-saline water, Na and Cl were highest in the root and the remaining elements were greatest in the stalk. The K and Cl contents were significantly reduced by an increase in the N level, whereas the reverse was true for the Ca, Mg and Na contents. An inverse relationship was noted for the plant biomass versus both Na uptake and the Na/Ca, Na/Mg and Na/K ratios in plants irrigated with saline water. The mineral elements, with the exception of K, appeared to be highly correlated in the plant parts.

Key words: saline water, nitrogen nutrition, mineral elements, partitioning

Introduction

The scarcity of good quality irrigation water is one of the major issues around the globe. The primary effect of saline water on crop productivity is the inability of the plant to compete with the ions in the soil solution for water (physiological drought). The higher the electrical conductivity (EC), the less water is available to the plants, even though a field may appear wet. The amount of water transpired through a crop is directly related to the yield; therefore, irrigation water with high EC reduces yield potential. Saline water resources are more abundant than fresh water. Bringing these resources into sustainable productive use will offer opportunities to increase food security especially in developing countries. Several physical, chemical and biological soil management measures promote the safe use of saline water in crop production. Some of these measures are tillage, deep ploughing, sanding, the use of chemical amendments and soil conditioners, organic and green manuring and mulching. The benefits expected from using soil management practices to facilitate the safe use of saline water for irrigation will not be realized unless adequate plant nutrients are supplied as fertilizers. Saline water irrigation along with nitrogen fertilization is essential for optimum crop productivity. The provision of nitrogen

has been reported to significantly mitigate the adverse effects of salts on several crops (Leidi et al., 1991). Attempts to enhance crop production under salinity through fertilizer management are of great significance. Previous studies have been focused mainly on the effect of salinity and nitrogen on plant growth and the uptake of mineral nutrients. The effect of saline water on the assimilation and partitioning of mineral elements and on their statistical relationship in maize plant parts supplied with nitrogen is poorly documented. The main objective of the present research was to compare saline and non-saline water interactions with the level of nitrogen on the distribution and correlation of Ca, Mg, Na, K and Cl uptake within the leaf, stalk and root of maize plants.

Materials and methods

A brief description of the physico-chemical characteristics of the soil used in this experiment was given in a previous report (Irshad et al., 2002). The soil was passed through a 4 mm sieve and two maize plants per pot were grown in Wagner pots filled with 3.5 kg soil for a period of six weeks. The plants were irrigated with either non-saline water (deionized water) or saline water (40 mmol l^{-1}). Saline water was prepared from a solution of $CaCl_2$, $MgSO_4$ and $NaHCO_3$ salts based on SAR 5 (i.e. equivalent to an EC value of 3.2 dSm^{-1}). The pH of the saline water was recorded as 7.6. The Ca/Mg ratio in the solution was 1:1 on a molar basis. According to Rhoades et al. (1992) such saline water is classified as moderately saline. The plants were subjected to three nitrogen levels, i.e. 50, 100 and 200 kg N ha^{-1} in the form of ammonium nitrate fertilizer, and a control (no N fertilizer) denoted as N1, N2, N3 and N0, respectively. Saline and non-saline water treatments were combined factorially with three levels of N and a control to give a total of eight treatments, arranged in a randomized complete block design with three replications in a greenhouse. A basal dose of 50 ppm each of P and K as $Ca(H_2PO_4)_2$ and K_2SO_4 (equivalent to 100 kg ha^{-1} for P and K) was applied to enhance crop growth. Initially the pots were irrigated with deionized water to avoid the deleterious effects of salinity on seedlings until the 2–3-leaf stage. Thereafter, the pots were irrigated with saline water up to field capacity by weighing the pots. Plant samples were thoroughly washed with deionized water and separated into leaf, stalk and root, then oven-dried to constant weight at 65°C, ground to pass through a 0.5 mm screen and digested in a mixture of HNO_3: H_2SO_4: $HClO_4$. The Ca, Mg, Na, K and Cl contents in the leaf, stalk and root were analysed according to the methods described earlier (Irshad et al., 2002a).

Statistical analyses

The data were statistically analysed using StatView software. Means and standard errors were calculated for three replicate values. The significance of differences between N treatments was further tested using Fisher's LSD test. Saline and non-saline treatments were compared for nutrient contents by the t-test at the 0.05% level of significance. The relationship between biomass yield and mineral content was determined using simple regression.

Results and discussion

The results for dry biomass and nitrogen recovery in maize (*Zea mays* L.) in this experiment have already been reported extensively elsewhere (Irshad et al., 2003). The statistical correlation and regression analyses for mineral elements (Ca, Mg, Na, K and Cl) and their partitioning within maize plant parts will be discussed below.

Calcium

The relationship between salinity and nutrient bioavailability is extremely complex and variable depending on the type of plant and the salt and nutrient contents in the soil. Significant changes were noted for Ca in response to saline water and nitrogen nutrition. Calcium levels (meq 100 g^{-1}) ranged from 4.7 in the root to 11.7 in the stalk for non-saline water, whereas for saline water the range was from 5.7 in the root to 13.9 in the leaf. These results showed the marked effect of water salinity on the partitioning of Ca content (Table 1). The calcium content in the leaf was not affected by ammonium nitrate nutrition, whereas the root and stalk had a higher content of Ca at increasing N rates. Among the N treatments (Table 2), the Ca content decreased in the order N3 (35.5) > N2 (26.7) > N1 (23.5) > N0 (22.7). It was also reported by Papadopoulos and Rendig (1983) that higher amounts of N applied to tomato plants significantly increased the concentrations of cations in the leaves under saline conditions. Previous findings also showed that in response to different forms of N (NH_4^+ and NO_3^-) the Ca concentration in maize shoots was enhanced as compared to the control (Irshad et al., 2002b). The cumulative values of Ca differed in the plant parts as follows: leaf > stalk > root (Table 2). Marschner (1995) also reported that Ca was preferentially localized in the leaf vacuoles as compared to other elements. Correlation coefficients (Table 3) showed that under salinity stress the Ca content was highly correlated with Mg and Na, but negatively related to the K content.

Magnesium

The magnitude of the effect of water salinity and N level on the Mg content in maize plants differed widely (Tables 1 and 2). The Mg content (meq 100 g^{-1}) was lowest (8.2) in the root and highest (11.7) in the stalk under normal water. For saline water the values were lowest (19.1) in the root and highest (33.0) in the leaf. The contents were significantly increased in plants fertilized with increasing amounts of N (ranging from 49.1 in N0 to 72.6 in N3). This could confirm that N fertilizer and saline water affected the distribution as well as the concentration of Mg in the leaf, stalk and root. An increase in the Mg content was noted in privet stalks as the N nutrition increased, whereas the concentration of Mg in the leaves was not affected (Stratton et al., 2001). Chaudhry and Khanif (2001) observed an increase in the Mg uptake by rice with increasing rates of N application. Kurvits and Kirkby (1980) reported that the form of N might have a considerable influence on the nutrient content of plants. The distribution and correlation pattern of Mg in the leaf, stalk and root with respect to other mineral elements was similar to that of Ca (see Table 3). The simple regression method (Table 4) showed that the Mg content (X) was related to plant dry biomass (Y) using the following equation: $Y = -4.03 + 0.13X$.

Table 1

Mean ($\overline{X}$), standard error (SE) and coefficient of variation (CV) of mineral elements (meq. 100 g^{-1}) in maize under non-saline and saline water, together with the results of the t-test

Plant parts	Non saline water			Saline water			t-test (0.05)	Level of significance
	$\overline{X}$	SE	CV (%)	$\overline{X}$	SE	CV (%)		
Ca								
Leaf	9.8	0.8	15.8	13.9	0.3	4.9	8.3	*
Stalk	11.7	2.6	43.8	8.3	0.8	19.0	1.8	NS
Root	4.7	1.1	45.1	5.7	0.5	16.9	1.5	*
Total	26.2	4.4	33.3	27.9	1.6	11.2	7.1	NS
Mg								
Leaf	12.6	0.6	7.4	33.0	3.5	21.5	6.5	***
Stalk	17.7	1.4	16.9	26.9	3.8	28.3	3.7	**
Root	8.2	0.9	22.4	19.1	1.4	15.0	9.4	***
Total	38.5	2.6	13.7	79.0	8.7	22.3	12.9	***
Na								
Leaf	0.5	0.1	26.7	29.9	1.8	12.3	16.0	***
Stalk	0.4	0.1	16.4	56.4	4.7	16.9	11.5	***
Root	35.8	2.3	12.8	116.2	2.1	3.7	20.9	***
Total	36.7	2.5	13.8	202.5	8.2	8.1	38.5	***
K								
Leaf	42.2	3.2	15.3	47.3	3.1	13.4	3.4	*
Stalk	49.1	9.4	38.7	66.6	2.4	7.4	3.1	NS
Root	19.5	5.1	52.7	11.7	3.1	53.5	3.3	*
Total	110.7	17.7	32.0	125.6	8.4	13.4	3.8	*
Cl								
Leaf	8.4	2.3	55.9	36.1	3.2	17.9	15.8	***
Stalk	8.1	6.6	64.2	83.7	2.7	6.5	14.9	***
Root	140.7	24.7	35.2	62.9	5.9	19.0	3.4	*
Total	157.6	42.2	27.2	182.7	7.7	8.4	7.3	**

*, ** and *** = significant at $P < 0.05$, <0.01 and <0.001; NS = not significant

Sodium

The results of the t-test showed that Na contents (meq 100 g^{-1}) increased markedly after the application of saline water (Table 1). In saline water the leaf exhibited the lowest (29.9) Na content, followed by the stalk (56.4) and the root (116.2) whereas for non-saline water the root had a higher level of Na than the leaf or stalk. The Na content was significantly increased in plants receiving the N3 treatment, whereas the N0, N1 and N2 treatments were statistically at par. The sodium content was highly correlated with other elements, with the exception of K (Table 3). There was a negative relationship between dry weight and Na uptake. This showed that the higher accumulation of Na in the plant could lead to lower biomass yield (Table 5). Plants fertilized with increasing amounts of N had lower Na/Ca and Na/Mg ratios. Galiba and Erdei (1986) and Trivedi et al. (1991) reported that nutritional imbalance was a growth-limiting factor in salt-stressed crops. The relationship between dry biomass (Y) and Na content (X) was described by the following regression equation: $Y = -17.82 + 0.20X$ (Table 4).

Table 2
Mineral element content (meq 100 g^{-1}) of maize plants as affected by levels of nitrogen

Nitrogen level	Leaf	Stalk	Root	Total
		Ca		
N0	10.8±2.3	7.6±0.2	4.3±1.0	22.7±3.1
N1	11.2±2.2	7.9±1.1	4.3±0.5	23.5±1.7
N2	12.2±2.1	9.7±1.0	4.7±0.9	26.7±2.0
N3	13.3±1.3	14.8±4.4	7.4±0.4	35.5±3.5
$LSD_{0.05}$	NS	5.5	1.5	6.5
		Mg		
N0	19.1±7.2	17.1±2.2	12.8±4.3	49.1±13.0
N1	19.6±7.7	19.4±2.7	11.8±4.7	50.9±15.2
N2	25.4±12.7	23.8±6.3	13.2±6.8	62.5±25.8
N3	26.9±13.1	28.9±7.0	16.7±6.1	72.6±26.3
$LSD_{0.05}$	4.3	5.8	NS	2.5
		Na		
N0	15.0±4.3	23.8±14.2	78.1±19.9	117.0±22.0
N1	14.8±8.5	27.2±16.3	74.2±18.2	116.3±25.2
N2	13.4±6.3	27.5±13.5	73.9±12.2	114.8±20.1
N3	17.8±4.1	34.9±15.8	77.7±14.4	130.5±18.6
$LSD_{0.05}$	2.4	5.7	NS	6.8
		K		
N0	53.5±1.7	73.2±3.2	27.1±7.7	153.9±8.5
N1	43.6±4.4	58.7±11.9	15.7±2.8	118.0±13.5
N2	42.9±3.1	50.7±15.2	9.8±1.6	103.4±16.6
N3	38.8±1.1	48.6±11.3	9.8±1.6	97.2±8.4
$LSD_{0.05}$	8.9	15.2	12.5	11.8
		Cl		
N0	19.5±3.9	58.6±7.1	131.1±15.6	209.2±26.9
N1	17.6±4.1	39.4±8.2	91.1±24.3	148.2±17.3
N2	22.2±6.1	40.7±9.8	68.7±19.3	131.6±36.5
N3	29.4±4.3	44.7±4.5	116.4±18.5	190.5±15.6
$LSD_{0.05}$	7.6	13.7	14.6	25.6

Table 3
Chart of correlation coefficients between mineral contents (meq 100 g^{-1}) in the leaf (L), stalk (S) and root (R) of maize plants under water salinity and N application

		Ca			Mg			Na			K			Cl	
		L	S	R	L	S	R	L	S	R	L	S	R	L	S
Ca	S	0.9													
	R	0.7	0.9												
	L	0.9	0.9	0.8											
Mg	S	0.9	0.9	0.8	0.9										
	R	0.9	0.9	0.9	0.9	0.9									
	L	0.4	0.6	0.7	0.3	0.5	0.6								
Na	S	0.8	0.8	0.8	0.7	0.9	0.8	0.7							
	R	0.8	0.9	0.9	0.8	0.9	0.9	0.7	0.8						
	L	–0.9	–0.8	–0.7	–0.8	–0.9	–0.8	–0.5	–0.9	–0.8					
K	S	–0.8	–0.9	–0.9	–0.9	–0.9	–0.9	–0.6	–0.9	–0.9	0.8				
	R	–0.9	–0.7	–0.6	–0.9	–0.9	–0.8	–0.3	–0.8	–0.7	0.9	0.8			
	L	0.8	0.9	0.9	0.9	0.9	0.9	0.5	0.8	0.9	–0.7	–0.9	–0.7		
Cl	S	–0.1	0.3	0.5	0.1	0.1	0.3	0.5	0.1	0.4	0.1	–0.3	0.3	0.4	
	R	–0.8	–0.5	–0.4	–0.6	–0.7	–0.5	–0.3	–0.7	–0.4	0.9	0.5	0.3	0.4	0.5

Table 4
Regression analyses for mineral content (X) in meq 100 g^{-1} and plant biomass (Y) in g pot^{-1} as affected by saline water and nitrogen nutrition

Variable (X)	Regression equation	R^2 value
Ca	Y = –11.12 + 0.63X	0.48
Mg	Y = –4.03 + 0.13X	0.67
Na	Y = –17.82 + 0.20X	0.49
K	Y = 26.– 0.15X	0.86
Cl	Y = 28.35 – 0.11X	0.42
Na/Ca	Y = 26.48 – 2.74X	0.14
Na/Mg	Y = 20.56 – 5.32X	0.63
Na/K	Y = –4.0 + 6.41X	0.66

Table 5
Correlation coefficient between the dry weight (g pot^{-1}) and uptake of mineral elements (mg pot^{-1}) in maize plants treated with saline water

Elements	Plant part	Leaf (L)	Stalk (S)	Root (R)	Total (T)
Ca	L	0.9	0.8	0.6	0.8
	S	0.9	0.8	0.5	0.8
	R	0.9	0.8	0.5	0.8
	T	0.9	0.9	0.6	0.9
Mg	L	0.4	0.4	0.1	0.3
	S	0.7	0.7	0.4	0.6
	R	0.2	0.3	0.1	0.2
	T	0.5	0.5	0.1	0.5
Na	L	–0.2	–0.1	–0.3	–0.2
	S	–0.2	–0.2	–0.4	–0.3
	R	–0.1	–0.1	–0.2	–0.1
	T	–0.1	–0.1	–0.3	–0.2
K	L	0.9	0.9	0.8	0.9
	S	0.5	0.6	0.5	0.5
	R	0.2	0.2	0.4	0.3
	T	0.8	0.8	0.8	0.8
Cl	L	0.3	0.2	–0.1	0.1
	S	–0.3	–0.3	–0.4	–0.4
	R	0.6	0.5	0.6	0.6
	T	0.7	0.6	0.5	0.7
Na/Ca	L	–0.6	–0.5	–0.7	–0.6
	S	–0.6	–0.6	–0.7	–0.6
	R	–0.7	–0.7	–0.7	–0.7
	T	–0.8	–0.7	–0.8	–0.8
Na/Mg	L	–0.7	–0.7	–0.8	–0.7
	S	–0.7	–0.6	–0.7	–0.7
	R	–0.8	–0.7	–0.6	–0.8
	T	–0.9	–0.8	–0.8	–0.9
Na/K	L	–0.4	–0.4	–0.6	–0.5
	S	–0.4	–0.4	–0.6	–0.5
	R	–0.1	–0.1	–0.3	–0.2
	T	–0.5	–0.5	–0.7	–0.6

Potassium

Nitrogen fertilizer and saline water had significant effects on the K content and on its relationship with other elements in the leaf, stalk and root of maize plants. Potassium levels (meq 100 g^{-1}) ranged from 19.5 in the root to 49.1 in the stalk for non-saline water and from 11.7 in the root to 66.6 in the leaf for saline water (Table 1). The K content in the leaf, stalk and root decreased significantly with an increase in N nutrition. Stratton et al. (2001) also reported a similar decrease in K concentration with an increasing level of N. The cumulative values of K differed in the plant parts in the order stalk > leaf > root (Table 2). Karlen et al. (1988) reported that the stalk was the main accumulation pool for the elements P and K in corn. Correlation analyses (Table 3) showed that the K content in the plant was negatively correlated with mineral elements after irrigation with saline water. This could be due to the fact that these elements (Ca, Mg and Na) were applied with the saline water. The K content in plant tissues was reduced by increasing Na salinity and a higher Na/Ca ratio in the root medium (Subbarao et al., 1990). A decrease in K uptake with increasing concentrations of salts (Khan et al., 1995) and the increasing availability of Ca, Mg and Na from added salts (Classen and Wilcox, 1974) have also been reported. Potassium was highly related to the dry biomass of maize under saline water conditions (see Table 4).

Chloride

Considerable differences were induced in the Cl content in plants by the use of saline and non-saline water. The t-test showed that saline water significantly raised the Cl content in the plants (Table 1). The chloride content (meq 100 g^{-1}) was found to be the highest in the root (140.2) in non-saline water, whereas the content was higher in the stalk (83.7) in saline water. The highest value of Cl in the leaf was recorded in the N3 treatment, while for the stalk and root the highest values were found in the control (N0). In the whole plant, the Cl content decreased up to the N2 treatment. Shaviv et al. (1990) reported a significant reduction in the Cl content in wheat at increasing N application levels. The cumulative values of Cl differed between the plant parts in the order root > stalk > leaf (Table 2). The dry biomass was inversely related to the Cl uptake, especially in the leaf and stalk. This indicated that shoot biomass was reduced by Cl accumulation in saline water. There is a consensus among authors that nutritional disorders occur in plants under saline conditions (Lauchli and Schubert, 1989; Grieve and Fujiyama, 1987). The coefficient of variation (CV) appeared to be decreased for all mineral elements in saline water as compared to non-saline water (Table 1).

These findings suggest that changes in the chemical composition and mineral distribution within maize plant parts were profoundly influenced by nitrogen nutrition and saline water. A higher level of relationship was found between the nutrients under saline water conditions. The amount of essential or non-essential elements absorbed by a crop could be related to its biomass yield. The inverse relationship between plant biomass and both Na uptake and Na/Ca, Na/Mg and Na/K ratios may suggest problems in the uptake of essential nutrients and their utilization by plants under saline stress conditions.

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RESPONSES OF SOYBEAN GENOTYPES TO INTERCROPPING WITH MAIZE IN THE SOUTHERN GUINEA SAVANNA, NIGERIA

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Field trials were conducted in the wet seasons of 1997 and 1998 at Makurdi, Otukpo and Yandev in the Southern Guinea Savanna ecological zone of Nigeria to study the responses of ten soybean genotypes to intercropping. The experiment was laid out in a randomised complete block design. The genotypes TGX 1807-19F, NCRI-Soy2, Cameroon Late and TGX 1485-1D had the highest grain yield. All the Land Equivalent Ratio (LER) values were higher than unity, indicating that there is great advantage in intercropping maize with soybean. The yield of soybean was positively correlated with the days to 50% flowering, days to maturity, plant height, pods/plant and leaf area, indicating that an improvement in any of these traits will be reflected in an increase in seed yield. There was a significant genotype × yield × location interaction for all traits. This suggests that none of these factors acted independently. Similarly, the genotype × location interaction was more important than the genotype × year interaction for seed yield, indicating that the yield response of the ten soybean genotypes varied across locations rather than across years. Therefore, using more testing sites for evaluation may be more important than the number of years.

Key words: soybean, intercropping, land equivalent ratio

Introduction

Although agricultural research originally focused attention on sole cropping and ignored the potential of intercropping (Willey and Osiru, 1972), there has been a gradual recognition of the value of this type of cropping system (Norman, 1972; Blade, 1992). A survey in Northern Nigeria by Norman (1974) reported 83% of the crop land to be in mixed cropping. The farmer's choice of intercropping is based on diversity of diet and income source, stability of production, reduced insect and disease incidence, efficient use of family labour and intensive production with limited resources (Francis et al., 1976). It has also been shown that intercropping produces higher and more stable yields in a wide range of component combinations (Ofori and Stern, 1987).

The plant breeding methodology that has been developed for monocultures may not be readily applicable to complex systems. Consequently, it is necessary to evaluate elite lines under intercropping systems before release, in order to identify varieties that are adaptable to intercropping systems. The study was therefore conducted to determine the adaptability of ten elite soybean lines in a mixture with maize.

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Materials and methods

Field experiments were conducted during the rainy seasons of 1997 and 1998 on the experimental fields of the University of Agriculture, Makurdi, the National Cereals Research Institute, Yandev substation, and the Benue State Agricultural and Rural Development Authority (BNARDA) Farm Centre, Otukpo. The experiment was laid out in a randomised complete block design (RCBD) with three replications. Ten genotypes of soybean (6 early and 4 medium) were grown in association with one variety of maize (Mega 4). These are the two maturity classes that have been found to yield well in the Southern Guinea Savanna zone of the country. The genotypes used (Table 1) were selected based on their good seed quality, seed longevity and high seed production.

Table 1
Genotype and maturity class of ten soybean cultivars used in the study

No.	Genotype	Maturity class
1	TGX 1485-1D	Early
2	Cameroon Late	Medium
3	TGX 1660-15F	Early
4	TGX 1807-19F	Medium
5	NCRI-Soy 2	Medium
6	TGX 1789-7F	Early
7	TGX 1681-3F	Early
8	TGX 1019-2EB	Early
9	TGX 1799-8F	Early
10	TGX 1805-31F	Medium

The experimental plot was ploughed, harrowed and ridged. During land preparation, 150 kg NPK/ha was applied broadcast to the entire experimental plot and an additional 30 kg N/ha from urea was applied to the maize crop 6 weeks after planting (WAP). Soybean was planted at the recommended population rate of 266,666 plants/ha by drilling on top of the ridges (0.75 m × 0.05 m per plant) in both the sole and intercrop systems, while maize was planted beside the ridges. Maize was planted at half the recommended plant density (0.75 m × 1 m × 2 plants). The seeds were dressed with Apron-plus 50DS (10% metalaxyl, 34% furaticurla, 6% carboxin) at the rate of 10 g/3 kg seed. Two hoe weedings were carried out at 3 and 6 WAP. Data collected from soybeans include days to 50% flowering, days to maturity, plant height at maturity, lodging, shattering, pods/plant, leaf area, nodulation and disease incidence (bacteria pustule, *Cercospora* and mosaic virus infections) as well as the grain yield of soybean and maize. Data for each trait were analysed using a randomised complete block design with the year effect as the random effect and genotypes and locations as the fixed effects.

Results

The mean squares for the combined analysis of ten soybean genotypes grown in mixtures with maize for two years over three locations are presented in Table 2. Highly significant mean squares were detected for the genotype, the genotype × location effects and the genotype × location × year interaction. There were no significant differences for the location effect.

Table 2
Sources of variation, degrees of freedom (d.f.), mean squares of yield, seven attributes and disease scores for ten genotypes of soybean intercropped with maize at three locations in Benue state in 1997 and 1998

Source of variation	d.f.	1	2	3	4	5
Replication/L×Y	12	0.069	2.41^{**}	3.29	966.17^{**}	0.449^{*}
Location (L)	2	5.875	1458.89	1174.84	345.88	0.231
Years (Y)	1	0.004	$1.66\times10^{11**}$	$1.53\times10^{7**}$	0.00	1.385^{*}
Genotype (G)	9	0.592^{**}	$3.82\times10^{9**}$	75.86^{*}	155.04	0.163
L×Y	2	0.124	4008.10^{**}	1690.97^{**}	1164.21^{**}	1.119^{**}
G×Y	9	0.087	$1.90\times10^{8**}$	235.77^{**}	$1.58\times10^{8**}$	0.471^{*}
G×L	18	0.433^{**}	$4.92\times10^{7**}$	196.91	$7.78\times10^{6**}$	0.294
G×L×Y	18	0.115^{*}	480.40^{**}	206.61^{**}	254.4^{**}	0.556^{**}
Error (Pooled)	108	0.050	0.852	2.33	15.30	0.220
	6	7	8	9	10	11
Replication/L×Y	0.44	16.26	0.144	0.112	0.498^{**}	0.124^{*}
Location (L)	0.595	615.86	0.54	0.017	3.217	0.101
Years (Y)	0.221	3139.18^{**}	0.267	0.071	13.228^{**}	0.41^{**}
Genotype (G)	0.529	419.39	0.260	0.031	0.117	0.035
L×Y	1.475^{**}	2472.86^{**}	0.780^{**}	0.109	12.032^{**}	0.136
G×Y	0.649	395.06	0.301^{*}	0.099	1.60	0.026
G×L	0.482	1039.69^{**}	0.336	0.137	0.618	0.069
G×L×Y	0.686^{**}	696.64^{**}	0.462^{**}	0.170^{*}	1.782^{*}	0.086^{**}
Error (Pooled)	0.194	22.2	0.116	0.094	0.196	0.004

1: Yield; 2: Days to 50% flowering; 3: Days to maturity; 4: Plant height; 5: Plant lodging; 6: Shattering; 7: Pods/plant; 8: Nodulation; 9: Bacterial pustule; 10: *Cercospora* infection; 11 Mosaic virus infection; * and **: significant at the p = 5% and 1% levels, respectively.

The yield of soybean was reduced by maize by 3–21%. The variety TGX 1807–19F in a mixture with maize had the highest yield of 1697 kg/ha in 1997 (Table 3) while it was third highest in 1998 (1452 kg/ha). The yield of sole maize was 2266 kg/ha, while the mean yield of maize in the intercrop ranged from 932 kg/ha to 1137 kg/ha. In 1997, there were significant differences between the soybean genotypes for yield and mosaic virus score. Similarly, the genotypes were highly and significantly different with regards to days to 50% flowering, days to maturity, plant height and shattering score. There were no significant differences between the genotypes with respect to lodging, number of pods/plant, leaf area, nodulation, bacterial pustule and *Cercospora* infections. Similar results were obtained in 1998 for all these attributes.

The Land Equivalent Ratio (LER) values are presented in Table 4. Each of the ten genotypes had higher total productivity under intercropping than as a sole crop. Consequently, all the LER values were higher than unity. TGX 1807-19F had the highest LER value of 1.40, though this was not significantly different from the other values.

Table 3
Mean yield (kg/ha) and coefficient of variation (CV %) of ten soybean genotypes intercropped with maize at three locations in Benue state in 1997 and 1998

Crop mixtures	1997		1998	
	Yield	CV (%)	Yield	CV (%)
TGX 1485-1D+maize	1310	31.8	1453	17.4
Cameroon Late+maize	1487	39.5	1325	14.8
TGX 1660-15F+maize	1090	44.4	1256	21.5
TGX 1807-19F+maize	1697	27.7	1452	31.6
NCRI-Soy2+maize	1553	2.7	1489	19.3
TGX 1789-7F+maize	1147	30.9	1111	25.3
TGX 1681-3F+maize	1097	18.2	1080	22.2
TGX 1019-2EB+maize	1053	21.3	1113	31.9
TGX 1799-8F+maize	1290	22.1	1195	20.6
TGX 1805-31F+maize	1193	2.7	1348	14.3

Table 4
Land equivalent ratio (LER) values for a soybean-maize intercrop at three locations over two years, 1997 and 1998

Genotypes	1997				1998			
	1	2		LER	1	2		LER
		S.	M.			S.	M.	
TGX 1485-1D	1900	1310	792	1.08	1513	1453	1229	1.45
Cameroon late	1727	1487	837	1.27	1602	1325	1313	1.30
TGX 1660-15F	1540	1090	820	1.12	1220	1256	1241	1.53
TGX 1807-19F	1857	1697	883	1.35	1475	1452	1177	1.45
NCRI-Soy2	1747	1553	996	1.38	1663	1489	1202	1.37
TGX 1789-7F	1520	1147	789	1.14	1049	1111	1131	1.51
TGX 1681-3F	1130	1097	1187	1.56	1340	1080	1087	1.24
TGX 1019-2EB	1273	1053	982	1.32	1156	1113	1119	1.41
TGX 1799-8F	1637	1290	806	1.19	1230	1195	1109	1.41
TGX 1805-31F	1847	1193	755	1.02	1456	1348	1112	1.37
Sole maize (Mega 4)	2024	–	–	–	2507	–	–	–

1: Sole crop yield; 2: Intercrop yield; S: Soybean; M: Maize

There were highly positive correlations between soybean seed yield and pods/plant, leaf area, plant height, days to maturity and days to 50% flowering. The relationship between yield and lodging score was also positive, but non-significant. The correlation between soybean yield, pod shattering, *Cercospora* infection, bacterial pustule and mosaic infection was negative and significant (Table 5).

Table 5
Correlation of soybean yield (kg/ha) with other plant traits in a soybean/maize intercrop

	1	2	3	4	5	6	7	8	9	10	11
Yield	0.516*	0.444	0.639*	0.104	–0.635*	0.746*	0.695*	–0.194	–0.489	–0.617*	–0.823*
1		0.968	0.809	–0.198	–0.842*	0.568	0.431	–0.260	0.034	0.085	–0.646*
2			0.785	–0.336	–0.764*	0.450	0.349	–0.173	0.173	0.231	–0.842*
3				–0.185	–0.679*	0.742*	0.438	0.051	–0.056	–0.134	–0.623*
4					–0.045	0.272	–0.306	–0.203	0.276	0.021	–0.149
5						–0.650*	–0.618*	0.408	0.424	0.012	0.707*
6							0.434	–0.255	–0.323	–0.129	–0.753*
7								0.062	–0.638*	–0.548*	–0.732
8									0.078	–0.323	0.065
9										0.577*	0.528*
10											0.512*
11											

1: Days to 50% flowering; 2: Days to maturity; 3: Plant height; 4: Plant lodging; 5: Pod shattering; 6: Pods/plant; 7: Leaf area; 8: Root nodulation; 9: Bacterial pustule; 10: *Cercospora* infection; 11: Mosaic infection; *Significant at the 5% level

Discussion

There were significant differences in the yields of the soybean genotypes. This makes it imperative to evaluate the genotypes in terms of total production to determine the best varieties for inter-cropping. Both maize and soybean suffered reduced yields when compared with their sole crop yields from an equivalent area of land. The genotypes TGX 1807-19F, NCRI-Soy2, Cameroon Late and TGX 1485-1D gave the highest yields. The leaf area was found to be positively correlated with soybean grain yield ($r = 0.69$) in the soybean/maize intercrop. A significant correlation between leaf area and yield was also observed by Shibles and Weber (1965). Therefore, the lower yield of soybean when intercropped with maize was mainly due to shading by the cereal crop, so that soybean growth was limited by reduced light intensity. Willey (1979) also reported that as the duration of a legume crop increases, competition for light becomes more severe.

The yield of soybean was positively correlated with days to 50% flowering, days to maturity, plant height, pods/plant and leaf area. Therefore, an improvement in any of these traits will be reflected in an increase in seed yield. This result agrees with the findings of Jacobs et al. (1984). The correlation coefficients obtained in both sole and intercrop situations were similar. This suggests that the correlation coefficients are consistent. The negative correlation between soybean yield and such traits as pod shattering, bacterial pustule, *Cercospora* and mosaic virus was to be expected.

Evidence from studies by Ezumah and McGuire (1982) suggests that the wider the difference in maturity date between intercropped maize and soybean,

the lower the competition for biomass growth between the component crops. Soybean and maize varieties that mature at the same time are competitive and mutually exclusive, so such a mixture should be used only when equal importance is attached to the yields of both crops. This suggests that differences in the maturity time and growth habits of the component crops are important determinants of the productivity of soybean intercrops, as found for other crop combinations. The maize variety used in this study had no adverse effect on the productivity of soybean, because there was more than 30 days difference in the maturity dates.

The productivity of the intercrop was assessed by means of LER, which indicated that there was a great advantage in intercropping maize with soybean, as evidenced by the fact that all the LER values were above unity. There is an indication that the elite soybean genotypes used in this study can maintain their high yield potential under intercropping with maize. Kamuanga et al. (1988) also reported that crop associations consistently gave higher total grain yields (LER > 1).

The LER values were not significantly different from each other. This may indicate that the climatic conditions of Benue State, located primarily in the Southern Guinea Savanna are suitable for the growth of both early and medium duration soybean genotypes in a mixture with maize. The 1997 results gave higher LERs, probably because there was more rainfall than in 1998, thereby providing more moisture for crop growth. This result supports the argument that genotypes selected for sole cropping may not be the best for intercropping (Fakorede and Obilana, 1979).

The combined analysis of variance showed significant differences for almost all the traits for genotype × year × location interactions. This suggests that none of these factors (genotype, year and location) acted independently. However, the genotypes responded differently, relative to each other, to a change in environment (location and year). Similarly, the genotype × location interaction was more important than the genotype × year interaction for seed yield. This suggests that the yield response of the ten soybean genotypes varied across locations rather than across the years. Thus, using more testing sites for evaluation may be more important than the number of years.

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FERTILISATION, RAINFALL AND CROP YIELD

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The effect of rainfall quantity and distribution and of N, P, K, Ca and Mg fertilisation on the yields of rye, potato, winter wheat and triticale were evaluated in the 42 years of a long-term mineral fertilisation experiment [soil (acidic, sandy, brown forest) × fertilisation (N, P, K, Ca, Mg) × rainfall (quantity, distribution) × crop (rye, potato, winter wheat, triticale)] set up in 1962 under fragile agro-ecological conditions in the Nyírlugos-Nyírség region of Eastern Hungary. The soil had the following agrochemical characteristics: pH (H_2O) 5.9, pH (KCl) 4.7, hydrolytic acidity 8.4, hy_1 0.3, humus 0.7%, total N 34 mg kg^{-1}, ammonium lactate (AL)-soluble P_2O_5 43 mg kg^{-1}, AL-K_2O 60 mg kg^{-1} in the ploughed layer. From 1962 to 1980 the experiment consisted of 2×16×4×4=512 plots and from 1980 of 32×4=128 plots in split-split-plot and factorial random block designs. The gross plot size was 10×5=50 m^2. The average fertiliser rates in kg ha^{-1} $year^{-1}$ were nitrogen 45, phosphorus 24 (P_2O_5), potassium 40 (K_2O), magnesium 7.5 (MgO) until 1980 and nitrogen 75, phosphorus 90 (P_2O_5), potassium 90 (K_2O), magnesium 140 ($MgCO_3$) after 1980. The main results and conclusions were as follows:

The rainfall quantities averaged over many years and in the experimental years, and during the growing season, averaged over many years and in the experimental years, were 567, 497, 509, 452 mm for rye and 586, 509, 518 and 467 mm for winter wheat.

Rainfall deviations from the many years' average –3% and –13% in the experimental years and during the growing season for potato and 2% and –3% for triticale.

During the vegetation period the relationships between rainfall quantity, NPKCaMg nutrition and yield could be characterised primarily by quadratic correlations. Maximum yields of 4.0 t ha^{-1} for rye, 21.0 t ha^{-1} for potato, 3.4 t ha^{-1} for winter wheat and 5.0–6.0 t ha^{-1} for triticale were recorded when the natural rainfall amounted to 430–500, 280–330, 449–495 and 550–600 mm, respectively. At values above and below these figures there was a considerable reduction in the yield.

The results showed that the crop yields were strongly influenced (quadratic correlation) by interactions between N, P, K, Ca and Mg fertilisation and rainfall quantity and distribution.

Key words: rainfall, crops, nutrient supply, yield

Introduction

The relationship between agricultural production and climate change is well established (Berényi, 1944; Szász, 1981; Adams et al., 1990; Harnos, 1993; Barrow et al., 2000; Downing et al., 2000; Bocz, 2001). There is, therefore, growing concern about the potentially wide-ranging impacts that climate change could have on these key factors as the nature and extent of anticipated changes have become more evident. These include changes in land use and in plant production and their management. These changes are unprecedented in terms of both their rate and their spatial extent. Changes in land use (agrotechnics, soil

cultivation, fertility, quality, protection, etc.) and in plant production (plant nutrition, rotation, protection, etc.) are currently the main manifestations of this (Stefanovits, 1966; Győrffy and Sváb, 1993; Várallyay, 1984; Kováts et al., 1985; Lásztity, 1991; Kádár, 1992; Kádár and Szemes, 1994; Németh, 1996). Due to its complexity it must be regarded as an interdisciplinary problem (Láng 1973; 2003).

Among the natural catastrophes, droughts and floods generally cause the greatest problems in field crop production (Gyuricza and Birkás, 2000). The droughts and floods experienced in Hungary in the early 1980s have drawn renewed attention to the analysis of these problems (Rácz, 1999).

Rye (*Secale cereale* L.), potato (*Solanum tuberosum* L.), winter wheat (*Triticum aestivum* L.) and triticale are demanding indicator crops of climate factors and soil nutrient status.

New research on climate change-soil-plant systems is focused on crop yields. This paper reports the rainfall change × soil × mineral fertilisation × plant interactions on crop yields in a long-term field experiment in Hungary.

Materials and methods

The effect of climate anomalies, especially the quantity and distribution of rainfall and mineral fertilisation (N, P, K, Ca, Mg), on rye, potato, winter wheat and triticale yields was examined from 1962 to 2001 in a long-term mineral fertilisation field experiment set up on an acidic, sandy, brown forest soil with alternating thin layers of clay substance in Nyírlugos, North-East Hungary.

The soil had the following agrochemical characteristics: pH (H_2O) 5.2–6.5, pH (KCl) 4.4–4.9, hydrolytic acidity 5.9–10.8, hy_1 0.2–0.4, humus 0.4–0.9%, total N 20.6–48.0 mg kg^{-1}, ammonium lactate (AL)-soluble P_2O_5 20–66 mg kg^{-1}, AL-K_2O 20–100 mg kg^{-1} in the ploughed layer.

From 1962 to 1980 the experiment consisted of 2 × 16 × 4 = 128 treatments in 4 replications, giving a total of 512 plots and from 1980 of 32 × 4 = 128 plots in split-split-plot and factorial random block designs. The gross plot size was 10 × 5 = 50 m^2. The fertiliser rates and combinations applied are shown in Table 1.

Nitrogen, in the form of 28% calcium ammonium nitrate, was applied in two equal splits in autumn and spring, while the P, K, Ca and Mg fertilisers were applied prior to ploughing in autumn in the form of 18% superphosphate, 60% potassium chloride, 95% limestone powder and 18% dolomite powder. In autumn 1997 the P, K, Ca and Mg fertilisers were applied for four years in advance. Rainfall data were estimated using the traditional Hungarian standard (Harnos, 1993) and crop-specific ecological standards (Márton, 2002a, b, c, d). The experimental databases were analysed by MANOVA and regression analysis (SPSS).

Results and discussion

Rye results between 1962 and 1972

The rainfall quantities averaged over many years and in the experimental years, and during the growing season, averaged over many years and in the experimental years, were 567, 497, 509 and 452 mm, respectively, for rye. On the basis of traditional (Harnos, 1993) and rye-specific rainfall deficiency values (Márton, 2002a) the years could be divided into average (1966), dry (1964, 1968, 1972) and wet (1970) years. Without fertilisation the weather anomalies

(drought, abundant rainfall) did not cause significant yield differences (average year: 1.63 t ha^{-1}, dry year: 1.51 t ha^{-1}, wet year: 1.47 t ha^{-1}). In the case of poor (30 kg ha^{-1}) N supplies the yields ranged between 2.35 and 2.77 t ha^{-1}. In the average year the yield was more than 1.0 t ha^{-1} higher than on the control plot, while dry and wet years led to yield reductions of 26 and 23%, respectively. With a moderate rate of N fertiliser (60 kg ha^{-1}) the yields were almost twice those in the control. The damaging effect of drought was reduced from 26% with poor N supplies to 15%. Excessive rainfall reduced the yields by 29%. At an N rate of 90 kg ha^{-1} the yields were greater than 3.5 t ha^{-1} in the average year, while this was reduced by 20% on average in the dry years and by 48% in the wet year. In general, close quadratic correlations could be demonstrated between the rainfall quantity during the vegetation period and the yield, depending on the fertilisation rate, with R values of 0: 0.9900***, N: 0.8400***, NP: 0.8400***, NK: 0.9100***, NPK: 0.8500***, NPKMg: 0.6500***. The best yields of around 4.0 t ha^{-1} were recorded when the natural rainfall amounted to 430–500 mm (Fig. 1). Rainfall quantities in excess of 500 mm caused severe yield reductions.

Table 1
Fertiliser rates (kg ha^{-1} $year^{-1}$) and combinations applied (Nyírlugos, 1962–2001)

Fertiliser rates

Control (no fertilisation)

Rate	Rye	Wheat	Potato	Rate	Rye	Wheat	Potato
N_1	30	30	50	P_2O_5	48	48	48
N_2	60	60	100	K_2O	80	80	150
N_3	90	90	150	MgO	15	15	30

N, P, K, Mg combinations

Control (no fertilisation)

N_1	N_2	N_3
N_1P	N_2P	N_3P
N_1K	N_2K	N_3K
N_1PK	N_2PK	N_3PK
N_1PKMg	N_2PKMg	N_3PKMg

Fertiliser rates from 1980

Level	N	P_2O_5	K_2O	$CaCO_3$	$MgCO_3$
Control	0	0	0	0	0
1	50	60	60	250	140
2	100	120	120	500	280
3	150	180	180	1000	-

N, P, K, Ca, Mg combinations

Control (no fertilisation)

N_1	N_2	N_3
N_1P	N_2P	N_3P
N_1K	N_2K	N_3K
N_1PK	N_2PK	N_3PK
N_1PKCa	N_2PKCa	N_3PKCa
N_1PKMg	N_2PKMg	N_3PKMg
$N_1PKCaMg$	$N_2PKCaMg$	$N_3PKCaMg$

Potato results between 1962 and 1979

The rainfall quantities deviated from the many years average in the experimental years and during the growing season of potato by –3% and –13%. The years were distinguished as dry (1973), wet (1965) or average (1963, 1967, 1969, 1971, 1975, 1977, 1979) on the basis of "general" (Harnos, 1993) and potato-specific (Márton, 2002b) rainfall deficiency limits.

The year effects in the experiments were determined chiefly by the rainfall quantities in the winter half-years, followed by the months prior to seeding, the vegetation periods, the summer half-years and the harvesting months. Without fertilisation the yield was reduced by 2.0–2.8 t ha^{-1} in the dry (1973) and wet (1965) years compared with the average years. At low nutrient rates (N, NP, NK, NPK and NPKMg combinations involving 50 kg ha^{-1} N) the yield was greater than in the control plots, especially in the dry year (8.0 t ha^{-1}), when averaged over the fertilisation treatments. In the average and wet years this value was 5.8 and 4.4 t ha^{-1}, respectively. Compared to the average years, the yield increase was around 67% in the dry year, while the yield in the wet year was similar to that in the average years. At medium nutrient rates (N, NP, NK, NPK and NPKMg combinations involving 100 kg ha^{-1} N) the maximum yields were produced in the NPK and NPKMg treatments. The year effects were more uniform due to the better nutrient status. In the average years the yield-increasing effect of fertilisation was over 7.0 t ha^{-1}. In the dry year this figure approached 10.0 t ha^{-1}, while in the wet year it dropped to 7.6 t ha^{-1}.

At high nutrient rates (N, NP, NK, NPK and NPKMg combinations involving 150 kg ha^{-1} N) the yield rose to over 16.0 t ha^{-1} when averaged over all the fertiliser treatments, which was 100% greater than that on the control plots. The yield-reducing effect of wet and dry years was not observed at this nutrient supply level, confirming the ability of fertilisation to compensate for poor weather conditions. The yield increased by 3% in the dry year and 12% in the wet year compared with the average. The positive effect of the wet year was almost 24% greater than at the medium nutrient level.

Close quadratic correlations were observed between the rainfall during the vegetation period and the yield, depending on the nitrogen rates (0: R=0.9800***, N: R=0.9500***) and on the NP (R=0.9600***), NK (R=0.9500***), NPK (R=0.9800***) and NPKMg (R=0.9600***) combinations. Yields close to the maximum (21.0 t ha^{-1}) were achieved in the 280–330 mm range (Fig. 2). Rainfall amounting to over 400 mm caused a substantial reduction in yield.

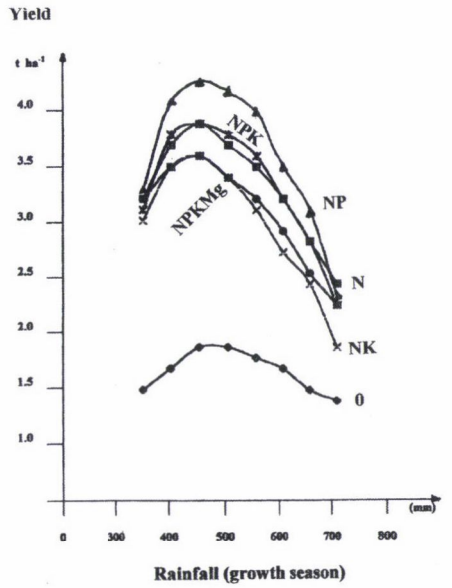

Fig. 1. Rainfall and fertilisation interactions on rye (*Secale cereale* L.) yield (Nyírlugos, 1962–1972)

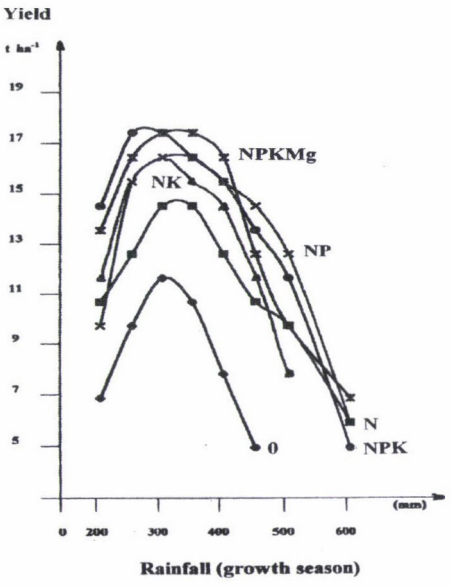

Fig. 2. Rainfall and fertilisation interactions on potato (*Solanum tuberosum* L.) yield (Nyírlugos, 1962–1979)

Winter wheat results between 1973 and 1990

The rainfall supplies to winter wheat over the average of many years and in the experimental years, and during the growing period, averaged over many years and in the experimental years, were 586, 509, 518 and 467 mm, respectively. On the basis of "general" (Harnos, 1993) and winter wheat-specific rainfall deficiency values (Márton, 2002c) the years could be classified as average (1978, 1982, 1989), dry (1974), droughty (1976, 1990) and wet (1980). In average years the yield of the control plots became stabilised at the 1.6 t ha^{-1} level. In the fertilised treatments the highest yield (3.7 t ha^{-1}) was more than one and a half times the lowest yield (2.3 t ha^{-1}).

N, NP and NK fertilisation resulted in an increase of around 1.0 t ha^{-1} in the main yield compared with the control. The wheat yields could only be enhanced economically by full treatment with NPK or NPKMg. Without fertilisation the yield in the dry year (1.7 t ha^{-1}) was similar to that in the average year (1.6 t ha^{-1}). The extent of loss was 12% in the N, NP and NK treatments and 10% in the NPK and NPKMg treatments. In the case of drought the grain yield of the control plots was approx. 30% lower than in the average year. The loss in plots given only N or the deficient NP and NK combinations was 41%, and this was aggravated by a further 7% in the NPK and NPKMg plots (48%). In the wet year the yield declined even more than in the case of drought. The unfertilised plots yielded over 80% less than in the average years.

In the case of unfavourable nutrition (N, NP, NK) the decrease in the harvested main yield was 64%, while the negative effect was slightly less (63%) in the NPK and NPKMg treatments. The relationships between rainfall during the vegetation period, N, P, K and Mg fertilisation and yield were characterised by second degree correlations (Fig. 3) depending on the level of nutrition (0: R=0.5949***, N: R=0.5734***, NP: R=0.7635***, NK: R=0.5357***, NPK: R=0.6710***, NPKMg: R=0.7055***). The grain yield per mm in the case of optimum rainfall supplies ranged from 3.7 to 7.2 kg ha^{-1}, depending on the fertiliser rate (0: 3.7, N: 4.6, NP: 6.1, NK: 4.8, NPK: 6.2, NPKMg: 7.2, treatment mean: 5.4 kg ha^{-1}). The natural rainfall was utilised better in the fertilised plots than in the untreated control (N: 24, NP: 65, NK: 28, NPK: 67, NPKMg: 95, treatment mean 46%). Supplementary magnesium fertilisation led to a 17% (1.0 kg ha^{-1} mm^{-1}) increase in yield compared to the NPK treatment. Maximum yields (3.4 t ha^{-1}) were recorded when the natural rainfall amounted to 449–495 mm.

Triticale results between 1990 and 2001

Rainfall discrepancies compared to the many years' average in the experimental years and during the growing season of triticale were 2% and –3%. On the basis of "general" (Harnos 1993) and specific rainfall supply values (Márton, 2002d) the years were characterised as average (1991, 1995, 2000), dry (1993), droughty (1992, 1994, 1996), wet (1997, 1998, 2001) and very wet (1999).

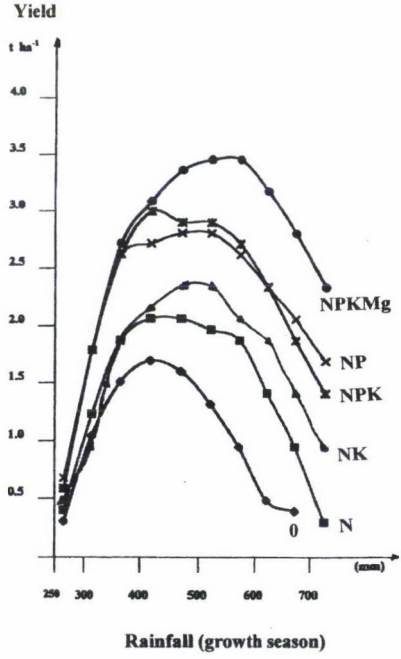

Fig. 3. Rainfall and fertilisation interactions on winter wheat (*Triticum aestivum* L.) yield (Nyírlugos, 1973–1990)

The year effects in the experiments were determined chiefly by the rainfall quantities in the winter half-year, the summer half-year and the month prior to sowing, and by the frequency of consecutive critical months during the vegetation period and the experimental year. In average years the yield of the unfertilised control plots was low (1.4 t ha^{-1}).

In the fertiliser treatments the maximum yield (4.0 t ha^{-1}) was more than twice the lowest yield (1.9 t ha^{-1}). N, NP and NK fertilisation resulted on average in a 1.0 t ha^{-1} yield increment compared to the control plots. The triticale yield could only be increased economically by the full NPK treatment (3.3 t ha^{-1}) or by combinations including calcium and magnesium (NPKCa, NPKMg, NPKCaMg) (3.9 t ha^{-1}). In dry and droughty weather the yield of the control areas was 14% and 36% less, respectively, than in average years. The application of N alone or of NP and NK treatments led to yield losses of 45 and 24%, respectively, while that of NPK, NPKCa, NPKMg or NPKCaMg caused a further 22% drop in both types of years. In the wet years the yield decreased by 14% in the unfertilised plots, remained unchanged in the case of N, NP or NK nutrition, and increased by 31% in the NPK, NPKCa, NPKMg and NPKCaMg treatments. In the very wet year the yields were similar to those in the average year. The relationships between rainfall quantity during the vegetation period, NPKCaMg nutrition and yield could be characterised primarily by quadratic correlations (Fig. 4. and 5) (R: Control = 0.3455***, N = 0.2779^{+}, NP = 0.4722***, NK = 0.3738***, NPK = 0.6311***, NPKCa = 0.6673***, NPKMg = 0.6734***, NPKCaMg = 0.6232***). Maximum yields in the region of 5.0–6.0 t ha^{-1} were achieved in the rainfall range of 550–600 mm, at around 580 mm. At values above and below this figure there was a considerable reduction in the grain yield.

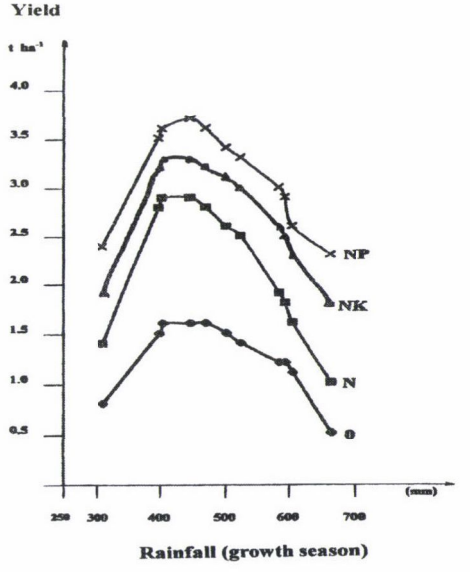

Fig. 4. Rainfall and fertilisation interactions on triticale yield (Nyírlugos, 1990–2001)

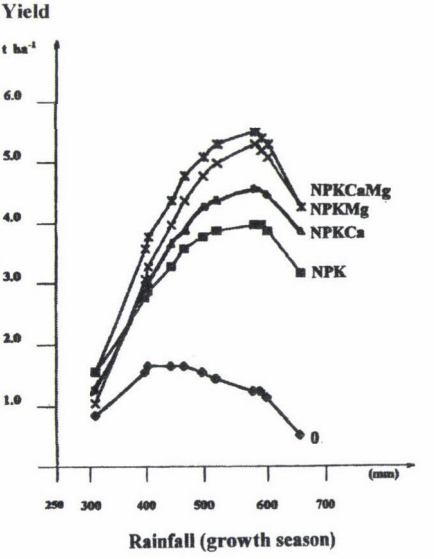

Fig. 5. Rainfall and fertilisation interactions on triticale yield (Nyírlugos, 1990–2001)

Acknowledgements

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NUTRIENT AVAILABILITY AND GRAIN YIELD OF WINTER SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH) AS INFLUENCED BY TILLAGE PRACTICES AND INTEGRATED NUTRIENT MANAGEMENT IN VERTISOLS OF SEMI-ARID TROPICS OF INDIA

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A field experiment was conducted in Vertisols at Bijapur during 1994–96 to study the effect of tillage practices and integrated nutrient management on winter sorghum yield and soil nutrient availability. The increase in winter sorghum yield with deep tillage over medium and shallow tillage was 27 and 57% in 1994–95 as compared to 18 and 34% in 1995–96. Deep tillage resulted in 22 and 45% higher yield as compared to medium and shallow tillage in the pooled data. This was mainly due to conservation and increased availability of moisture and nutrients, i.e. N, P and K. The higher availability of nutrients in the topsoil (0–0.15 m) as compared to the subsoil (0.15–0.30 m) was due to the application of nutrients in the topsoil layer and the higher rate of mineralization. Among the organic materials applied, *Leucaena* loppings at 2.5 t ha^{-1} led to a significantly (9%) higher yield (1636 kg ha^{-1}) over vermicompost (1500 kg ha^{-1}) and was on par with farmyard manure (1572 kg ha^{-1}) in the pooled data and during both years of the study. The higher percentage increase in grain yield with *Leucaena* application was due to the better moisture conservation and availability of major nutrients, i.e. N, P and K. Winter sorghum responded significantly to N application at 25 kg ha^{-1} in 1994–95, whereas in 1995–96 and in the pooled data the response varied up to 50 kg N ha^{-1}. In the pooled data, the grain yield increased by 17 and 24% with the application of 25 and 50 kg N ha^{-1} compared with the control. The higher yields obtained with the application of nitrogen were due to the better availability of nutrients, especially N, as these soils are low in available N.

Key words: tillage practices, organic materials, nitrogen, nutrient availability, sorghum

Introduction

The indiscriminate use of land, with faulty land and crop management practices, results in the loss of nearly 6000 Mt of fertile top soil (16.35 t ha^{-1}) through erosion every year, causing soil degradation in India. In addition to soil degradation, the storage capacity of reservoirs is reduced by 1 to 2% per year, thereby adversely affecting power generation, the shortage of which was greatly felt in recent times in India. In India, black soils occupy 73.0 million ha and soil loss has been estimated to be 23.7 to 112.5 t per ha every year, with agriculture as the main land use (Dhruva Narayana, 1986). Such loss of topsoil results in reduced moisture storage, nutrient depletion and increased runoff, eventually making farming uneconomical. Adopting suitable moisture conservation practices, including proper tillage practices, in drylands increases the water

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availability to crops, reduces the water and nutrient losses, and thereby stabilizes/sustains crop yields in the long run, especially during drought years. In addition, these soils are low in available N and low to medium in phosphorus, but due to a rise in the prices of raw materials, the increased use of fertilizers by farmers, especially in drylands, is unlikely. This has resulted in the persistent nutrient depletion of the soils, posing a great threat to sustainable agriculture. Organic manures (crop residues, twigs of trees) and compost have been used as a means of maintaining and increasing soil fertility throughout the history of farming. In recent times, the recycling of organic manure is an important aspect of environmentally sound, sustainable agriculture. Returning residues to the soil is vital for maintaining soil organic matter, which in turn improves the soil structure, soil and water conservation, and soil microbial and fauna activity (Unger, 1978). Appropriate tillage practices, with a judicious combination of chemical fertilizers, organic manures and biofertilizers, not only improve the physico-chemical and biological properties of the soils, but also improve the use efficiency of applied fertilizers, giving an increase in crop yields on a sustainable basis. In view of the above situation, the present experiment was conducted to study the effect of tillage practices and integrated nutrient management on the nutrient availability and grain yield of winter sorghum in Vertisols of semi-arid tropics in South India.

Materials and methods

A field experiment was conducted on lands having a 1.0% slope at the Regional Agricultural Research Station, Bijapur (16° 49' N and 75° 42' E) during the winter seasons of 1994–95 and 1995–96 in deep black soils (typic–Chromusterts). The experimental site received a mean annual rainfall of 649.9 mm distributed over 54 rainy days in 1993–1994, as against a total rainfall of 585.8 and 629.4 mm distributed over 36 and 47 rainy days with highest mean monthly rainfall figures of 339.5 mm (October) and 238.4 mm (September), in 1994–95 and 1995–96, respectively. During the 3rd week of June 1995, soil samples were collected from the experimental site and analysed for physico-chemical properties. The soils of the experimental site were alkaline in reaction (pH 8.5) with E.C. 0.27 dS m^{-1}, and were low in organic carbon (3.6 kg m^3), available N (128 kg ha^{-1}) and available P (12.3 kg ha^{-1}) and medium in available K (434 kg ha^{-1}). The experiment was laid out in a split-split plot design in three replications. Tillage treatments were imposed in the main plots during the 3rd week of June in 1995 and the 3rd week of May in 1996. Deep tillage to a depth of 0.30 m was carried out using a tractor-drawn mould board plough, medium tillage to a depth of 0.15 m with a bullock-drawn mould board plough and shallow tillage to a depth of 5 cm with a bullock-drawn harrow. Organic materials were applied in the sub-plots. *Leuceaena* loppings (*Leucaena leucocephala* Lam.) at 2.5 t ha^{-1} and farmyard manure at 2.5 t ha^{-1} were applied in the 3rd week of August and the 1st week of September, respectively, and covered manually. Vermicompost at 1.0 t ha^{-1}, nitrogen fertilizer as urea in the sub-sub plots (at 0, 25 and 50 kg ha^{-1}) and the recommended dose of phosphorus (25 kg ha^{-1}) were applied to all the treatments at the time of sowing. Maladandi M35–1, a winter/*rabi* sorghum cultivar, was sown on October 5 and September 14 to a depth of 5 cm, 0.15 m apart in 0.60 m rows and harvested on February 15, 1995 and January 25, 1996, respectively. After harvest, soil samples were collected from 0–0.15 and 0.15–0.30 m soil depths in all three replications and chemically analysed for available nitrogen by the alkaline permanganate oxidation method (Subbaiah and Asija, 1956), available phosphorus by Olsen's method (Jackson, 1967) and available potassium by extraction with neutral normal ammonium acetate and photometric estimation (Muhr et al., 1965). The data were analysed using the MSTAT–C package (Gomez and Gomez, 1984).

Results and discussion

Tillage practices

Deep tillage gave a significantly ($P<0.05$) higher grain yield than medium or shallow tillage in 1994–95, 1995–96 and in the pooled analysis. The increase in the grain yield in deep tillage compared with medium and shallow tillage was higher in 1994–95 (27 and 57%), when the crop was sown late (October 5) and drought occurred from flowering to the grain filling stage, than in 1995–96 (17.5 and 34%) when there was a uniform distribution of rainfall during the cropping season (Table 1). The higher sorghum yields achieved with deep tillage as compared to medium and shallow tillage were due to the better moisture conservation in the soil profile at sowing, 60 days after sowing and at harvest (Table 2). It is often tacitly accepted that soil moisture is the most limiting factor for crop production in drylands. The soil moisture in the profile at sowing ultimately determines the yields of post-rainy season (winter/rabi) crops. Higher yields of sorghum in deep tillage as compared to shallower depths of tillage were reported earlier in black soils in India at Sholapur (Anderson, 1980), Bellary (Anon., 1980), Dharwad (Rangaswamy, 1984) and Bijapur (Surkod, 1993), in sandy clay loam soils (Xerofluent) in Spain (Moreno et al., 1997) and in sandy clay loams in Pakistan (Ishaq et al., 2001). The response of straw yield to different tillage practices was similar to that of the grain yield, with deep tillage resulting in 17 and 33% higher yield than medium or shallow tillage.

Table 1

Grain and straw yields of winter sorghum as influenced by tillage practices, organic materials and nitrogen application

Treatment	Grain yield (kg ha^{-1})			Straw yield (t ha^{-1})		
	1994–95	1995–96	Pooled	1994–95	1995–96	Pooled
Tillage practices						
Deep tillage	1919	1835	1877	2.09	2.12	2.10
Medium tillage	1509	1562	1535	1.69	1.91	1.78
Shallow tillage	1223	1368	1296	1.48	1.68	1.58
S.Em.±	42	47	32	0.04	0.07	0.04
CD (P=0.05)	164	186	103	0.16	0.29	0.14
Organic materials						
FYM at 2.5 t ha^{-1}	1540	1604	1572	1.74	1.92	1.83
VC at 1.0 t ha^{-1}	1487	1512	1500	1.67	1.83	1.75
LEU at 2.5 t ha^{-1}	1625	1647	1636	1.84	1.96	1.90
S.Em.±	51	40	28	0.05	0.04	0.03
CD (P=0.05)	119	126	82	0.14	n.s.	0.09
Nitrogen application (kg ha^{-1})						
0	1375	1410	1393	1.57	1.71	1.64
25	1620	1595	1607	1.82	1.91	1.87
50	1657	1759	1708	1.86	2.09	1.97
S.Em.±	27	33	21	0.03	0.04	0.04
CD (P=0.05)	78	98	59	0.09	0.12	0.07

Note: FYM=Farmyard manure, VC=Vermicompost, *LEU=Leucaena* loppings, n.s.=Non-significant

Table 2
Soil moisture content in top 0.60 m as influenced by tillage practices, organic materials and nitrogen application

Treatments	At sowing		60 DAS		At harvest	
	1994–95	1995–96	1994–95	1995–96	1994–95	1995–96
Tillage practices						
Deep tillage	20.1	14.7	16.8	17.6	15.0	13.7
Medium tillage	19.7	14.2	16.1	17.2	13.5	13.7
Shallow tillage	19.7	13.3	15.0	16.5	12.5	13.4
CD (P=0.05)	0.31	0.94	0.86	1.06	1.37	n.s.
Organic materials						
FYM at 2.5 t ha^{-1}	19.7	14.2	16.1	17.2	13.6	13.6
VC at 1.0 t ha^{-1}	19.6	13.9	16.0	16.7	13.3	13.4
LEU at 2.5 t ha^{-1}	20.1	14.1	15.9	17.4	14.0	13.8
CD (P=0.05)	0.45	0.25	n.s.	n.s.	0.62	n.s.
Nitrogen application (kg ha^{-1})						
0	20.0	14.1	15.9	18.1	13.9	14.1
25	19.8	14.0	16.0	16.9	13.7	13.4
50	19.7	14.1	16.1	19.3	13.4	13.2
CD (P=0.05)	n.s.	n.s.	n.s.	0.69	0.49	0.29

Note: FYM=Farmyard manure, VC=Vermicompost, *LEU=Leucaena* loppings, n.s.=Non-significant, DAS = days after sowing

In addition to soil moisture, the availability of nutrients in the soil profile determines the crop yields in the Vertisols of drylands in the semi-arid tropics of South India, as these soils are not only thirsty but also hungry. Significantly higher available N was observed in the topsoil (0–0.15 m) and subsoil (0.15–0.30 m) at harvest during both years of the study in deep-tilled plots as compared to medium- and shallow-tilled plots. Available phosphorus increased significantly in deep tillage as compared to shallower depths of tillage in the first year alone (0–0.15 m and 0.15–0.30 m) and available K in the second year (0.15–0.30 m) (Tables 3, 4 and 5). The lower nutrient availability in the subsoil than in the topsoil was probably due to the lower organic matter content, whereas the higher availability of nutrients in the topsoil was due to the addition of nutrients manually. The surface application of fertilizers may result in greater losses of N by ammonia volatilization and in a higher amount of nutrient loss in surface runoff as compared to the incorporation of fertilizers annually by ploughing. Added to this, deep tillage increased the mineralization rate as compared to shallower depths of tillage, thereby increasing the availability of nutrients. Jayaram et al. (1982) stated that contour cultivation alone reduced the soil loss from 12.5 to 2.0 t ha^{-1} compared with up and down cultivation, thus reducing the nutrient losses (total N, available P and K and exchangeable Ca and Mg) in the Vertisols of Bellary (India). Similarly in the Vertisols of Akola (India), contour cultivation increased the residual N by 39 kg ha^{-1}, available P by 4.6 kg ha^{-1} and available K by 42 kg ha^{-1} over cultivation along the slope (Sagare et al., 1992). Earlier studies on tillage by Bhushan et al. (1979) and Larvea and Unger (1995) also support the results of the present investigation.

Table 3
Available soil nitrogen (kg ha^{-1}) at different soil depths at harvest in both years as influenced by tillage practices, organic materials and nitrogen application

Treatments	Soil depths			
	1994–95		1995–96	
	0–0.15 m	0.15–0.30 m	0–0.15 m	0.15–0.30 m
Tillage practices				
Deep tillage	141.4	135.4	154.3	144.3
Medium tillage	131.8	124.8	141.9	132.7
Shallow tillage	126.4	122.1	137.8	132.8
S.Em.±	0.9	0.8	1.5	1.4
CD (P=0.05)	3.6	3.1	5.9	5.3
Organic materials				
Farmyard manure at 2.5 t ha^{-1}	132.8	126.8	142.8	134.4
Vermicompost at 1.0 t ha^{-1}	129.7	124.3	142.1	133.4
Leucaena loppings at 2.5 t ha^{-1}	137.1	131.2	149.1	142.1
S.Em.±	1.6	1.7	3.3	2.0
CD (P=0.05)	5.0	5.1	n.s.	6.2
Nitrogen application (kg ha^{-1})				
0	121.5	116.4	129.7	122.3
25	133.1	127.9	145.3	138.4
50	145.1	138.0	158.9	149.2
S.Em.±	2.0	1.9	1.7	2.0
CD (P=0.05)	5.9	5.4	5.0	5.8

n.s.= non-significant

Table 4
Available soil phosphorus (kg ha^{-1}) at different soil depths at harvest in both years as influenced by tillage practices, organic materials and nitrogen application

Treatments	Soil depths			
	1994–95		1995–96	
	0–0.15 m	0.15–0.30 m	0–0.15 m	0.15–0.30 m
Tillage practices				
Deep tillage	29.0	27.7	33.0	29.9
Medium tillage	28.1	26.0	32.1	28.9
Shallow tillage	26.7	24.7	30.7	27.8
S.Em.±	0.3	0.4	0.7	0.7
CD (P=0.05)	1.0	1.4	n.s.	n.s.
Organic materials				
Farmyard manure at 2.5 t ha^{-1}	27.8	26.4	31.8	28.7
Vermicompost at 1.0 t ha^{-1}	27.1	25.8	31.1	27.8
Leucaena loppings at 2.5 t ha^{-1}	29.0	26.2	33.0	30.1
S.Em.±	0.8	0.5	0.9	0.7
CD (P=0.05)	n.s.	n.s.	n.s.	2.0
Nitrogen application (kg ha^{-1})				
0	27.6	25.9	31.6	28.4
25	27.7	26.2	31.7	28.4
50	28.6	26.3	32.6	29.7
S.Em.±	0.4	0.6	0.7	0.4
CD (P=0.05)	n.s.	n.s.	n.s.	1.3

n.s.= non-significant

Table 5
Available soil potassium (kg ha^{-1}) at different soil depths at harvest in both years as influenced by tillage practices, organic materials and nitrogen application

Treatments	Soil depths			
	1994–95		1995–96	
	0–0.15 m	0.15–0.30 m	0–0.15 m	0.15–0.30 m
Tillage practices				
Deep tillage	487	459	504	470
Medium tillage	464	433	483	448
Shallow tillage	441	417	457	432
S.Em.±	18.6	13.3	17.2	7.4
CD (P=0.05)	n.s.	n.s.	n.s.	29.1
Organic materials				
Farmyard manure at 2.5 t ha^{-1}	467	441	484	455
Vermicompost at 1.0 t ha^{-1}	448	415	463	430
Leucaena loppings at 2.5 t ha^{-1}	477	452	497	465
S.Em.±	12.3	8.7	12.1	11.5
CD (P=0.05)	n.s.	26.8	n.s.	n.s.
Nitrogen application (kg ha^{-1})				
0	430	419	446	435
25	481	443	499	455
50	481	447	500	461
S.Em.±	11.3	9.0	11.5	9.1
CD (P=0.05)	32.6	26.0	33.2	n.s.

n.s.= non-significant

Organic materials

The application of *Leucaena* loppings increased the grain yield by a significant 9% (1636 kg ha^{-1}) over vermicompost (1500 kg ha^{-1}) and was on par with farmyard manure (1572 kg ha^{-1}) in the pooled data and during both years of the study (Table 1). The straw yield increased significantly (8%) after *Leucaena* application (1.90 t ha^{-1}) as compared to vermicompost (1.75 t ha^{-1}). Earlier studies also indicated higher yields with *Leucaena* application as compared to the other organic materials used (Bellakki and Badanur, 1993; Durgude and Patil, 1997). The higher yields obtained with *Leucaena* application as compared to farmyard manure or other crop residues was due to the lower C:N ratio, the faster rate of mineralization, better moisture conservation and the higher availability of N, P and K during both years of the studies in the 0–0.15 and 0.15–0.30 m soil depths (Tables 3, 4 and 5). Higher available N was observed in the topsoil (0–0.15 m) as compared to the subsoil (0.15–0.30 m). In the second year, *Leucaena* application led to higher available N of 149.1 kg ha^{-1} in the topsoil as compared to farmyard manure and vermicompost (142.8 and 142.1 kg ha^{-1}). A similar trend was observed in the first year, when the differences were significant. In the subsoil, *Leucaena* application significantly increased the available N compared with the other organic materials. Available P and K were higher with *Leucaena* incorporation as compared to other organic materials during both years of the study in the top and subsoil. These results are in

conformity with the findings of Seth Jagadish and Balyan (1989), Badanur and Malabasari (1995), Durgude et al. (1996), Patil et al. (1996) and Shelke et al. (1997).

Nitrogen application

The grain and straw yields of winter sorghum increased significantly in both years of the study and in the pooled data, except for the straw yield in 1994–95, which only increased significantly up to 25 kg N ha^{-1}. In the pooled data, the application of 25 kg N ha^{-1} increased the grain yield by 17% (1607 kg ha^{-1}), while a further increase in N application to 50 kg ha^{-1} increased the grain yield by 24% (1708 kg ha^{-1}) over the control (1393 kg ha^{-1}) (Table 1). A similar trend was observed for the straw yield. The increase in the crop yield with an increase in N application to 50 kg ha^{-1} was due to the increased availability of N, P and K in the 0–0.15 and 0.15–0.30 m layers, resulting in an increased uptake of N, P and K (Tables 3, 4 and 5). An increase in the N application from 0 to 50 kg ha^{-1} increased the N availability from 129.7 to 158.9 kg ha^{-1} in the topsoil in 1995–96, a similar trend being observed in 1994–95 and in the subsoil, where the values were lower than in the topsoil (Table 3). The increased availability of N was due to the increased supplementation of N through fertilizer and possibly partly to mineralisation. Similarly, the marginal increase in the P and K contents in the 0–0.15 and 0.15–0.30 m soil layers after an increase in N application to 50 kg N ha^{-1} in both years of the study could be attributed to mineralisation (Tables 4 and 5). The results of the present studies are in accordance with the findings of Surkod (1993) and Shelke et al. (1997). The grain yield was positively correlated with the available N at the 0–0.15 m (r=0.536) and 0.15–0.30 m depths (r=0.518) and with the available K at the 0–0.15 m (r=0.511) and 0.15–0.30 m depths (r=0.512). In both years of the study an increase in N application from 0 to 25 and 25 to 50 kg ha^{-1} increased the N availability in the topsoil significantly. A similar trend was observed in the subsoil, with lower values in the first year (1994–95). The available P and K in the soil were higher at 50 kg N ha^{-1} than at 25 kg N or no N application. The higher availability of N, P and K in the soil was due to the higher amount of N added through fertilizer or organic materials, that may have increased the nutrient status of the soil, and to the mineralisation occurring in the soil. The above results are in conformity with the findings of Mastiholi (1994).

Interactions

The grain yield of winter sorghum differed significantly due to the interaction of tillage practices and nitrogen application in 1994–95 and in the pooled data. During 1994–95, the significant response to applied nitrogen (25 kg ha^{-1}) varied with medium (1576 kg ha^{-1}) and deep tillage (2047 kg ha^{-1}). In the pooled data, a response was observed up to 50 kg N ha^{-1} in deep tillage (2074 kg ha^{-1}) and up to 25 kg N ha^{-1} in medium (1577 kg ha^{-1}) and shallow tillage (1307 kg ha^{-1}) (Table 6). At the same level of nitrogen with different tillage practices, deep tillage always produced a significantly higher yield than medium or shallow tillage in 1994–95 and in the pooled data. In the pooled data, deep tillage with 50 kg N ha^{-1} gave a significantly higher grain yield (2074 kg ha^{-1}) than the other treatments, except 25 kg N ha^{-1} (1937 kg ha^{-1}).

Table 6
Grain yield of sorghum (kg ha^{-1}) as influenced by the interaction effects of tillage and nitrogen application

Tillage practices	1994–95				1995–96				Pooled			
Nitrogen application (kg ha^{-1})	0	25	50	Mean	0	25	50	Mean	0	25	50	Mean
Deep tillage	1631	2047	2080	1919	1610	1828	2068	1835	1620	1937	2074	1877
Medium tillage	1343	1576	1609	1509	1404	1578	1702	1562	1373	1577	1656	1535
Shallow tillage	1152	1236	1282	1223	1219	1378	1508	1368	1185	1307	1395	1296
Mean	1375	1620	1657	1551	1410	1595	1759	1588	1393	1607	1708	1569
Comparing means	S.Em±		$CD_{P=0.05}$		S.Em±		$CD_{P=0.05}$		S.Em±		$CD_{P=0.05}$	
N at same T	47.0		135.7		57.4		n.s.		37.1		102.9	
T at same/different N	56.7		163.7		66.6		n.s.		43.8		113.6	

Note: T= Tillage practices, N= Nitrogen application, n.s.= Non-significant.

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TECHNOLOGICAL QUALITY OF SPRING WHEAT LINES AT DIFFERENT RAINFALL LEVELS

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Due to developments in the food and baking industry, grain quality determines prices and market options to a large extent. The introduction of high quality wheat varieties into cultivation requires not only favourable technological parameters, but also good adaptation to unfavourable environmental conditions. The level of rainfall in Poland during the spring and summer differs greatly from one years to the other, so the varieties introduced into cultivation must be capable of giving high values of quality parameters with both an excess and deficit of rainfall. The aim of the present work was thus to study whether the quantity of rainfall affected the technological traits determining the industrial usefulness of the crop, and if so, in what way.

Interactions were observed between the evaluated genotypes and the environmental conditions (particular years and locations), which greatly influenced the average level of the technological traits. This was most strongly observed for traits related to gluten quantity and quality. The rainfall level over the whole vegetation period was not correlated with the technological traits examined, while the rainfall measured in May significantly influenced the sedimentation value and water absorption ($r= -0.68^{**}$ and $r= -0.54^{*}$), which are the traits most strongly related to the gluten quality and rheological qualities of the dough.

Key words: drought, spring wheat, technological quality

Introduction

The occurrence of drought limits plant growth and productivity to a much larger extent than any other factor, and annual profits from the production of crops on dry territories are only half those obtained with optimum irrigation (Rajaram, 2001). A lack of water for growing and maturing crops has always been a problem in Mediterranean zones. In recent years, however, this problem has started to affect countries with a temperate climate (Foulkes et al., 2001). It was reported by Zagdańska (1997) that in Poland losses caused by water deficiency in spring wheat crops amounted to 30% in some years. High yields are not, however, the sole element accounting for cultivation profitability. As a result of developments in the food and baking industries, grain quality now determines the sales price and marketing possibilities. It has therefore become necessary to introduce into cultivation quality forms of wheat which have satisfactory technological parameters even when there is a deficiency or excess of rainfall.

Drought is a multidimensional stress affecting most of the physiological and biochemical processes taking place during plant growth and development.

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Water deficiency affects the energy metabolism of the plant, decreases the activity of some enzymes and increases the accumulation of osmoprotectants in the cells (Zagdańska, 1995; Szegletes et al., 2000; Chandrasekar et al., 2000). The level of chlorophyll a and b drops, as well as that of total chlorophyll, which, in turn, decreases the efficiency of photosynthesis (Nyachiro et al., 2001). This affects basic flour ingredients, by lowering the protein and starch contents and enzyme activity (Cygankiewicz, 1995).

This phenomenon could be expected to affect quality parameters. Therefore, while trying to satisfy consumer needs and the requirements imposed by industry, it is necessary to examine a large number of forms and to make an appropriate choice of the most suitable lines. The practical significance of looking for new quality forms has motivated many authors to investigate this subject (Węgrzyn et al., 1992; Szwed-Urbaś, 1992; Bichoński, 1995; Cygankiewicz, 2000). The aim of the present work was to observe whether and in what degree the rainfall level influenced the values of the technological traits determining the industrial usefulness of spring wheat grain.

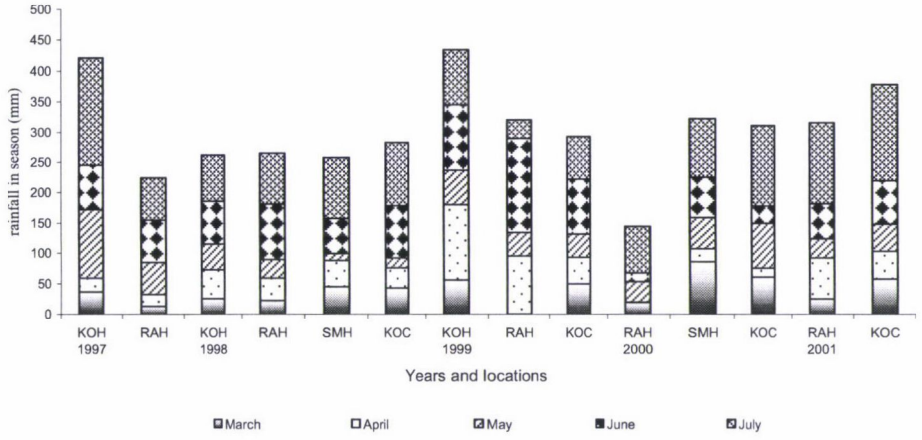
Materials and methods

The experimental material consisted of spring wheat lines in generations F_6–F_7 evaluated in breeding experiments at Breeding Stations in Kończewice (KOH), Pustków (KOC), Radzików (RAH) and Smolice (SMH) in the period between 1997 and 2000. The set of genotypes and locations changed from year to year according to the principles accepted for cultivation experiments. However, the investigated lines were the same at all locations in any given year. In all the locations the lines were grown on plots with an area of 10 m^2, with the standard level of fertilisation, i.e. 70, 100 and 150 kg/ha N, P, K, respectively. The experiments were carried out in a randomized block design with four replications

In order to unify the results and facilitate comparisons, the rainfall level at all the locations was measured from the beginning of March till the end of July. In each year, grain was collected at 2–4 locations and the following traits were recorded: sedimentation value, falling number, protein content, water absorption, quality number, degree of softening, wet gluten content and gluten index, bread volume, estimated bread crumb and grinding, in accordance with the method described by Cygankiewicz (2000).

Results and discussion

Figure 1 shows the seasonal and monthly sums of rainfall recorded at the locations from which the grain samples were collected for technological analysis. Working on the assumption that, in the case of wheat, a rainfall level of 400 mm per season guarantees a satisfactory water supply for cultivation, whereas 300 mm is insufficient (Uddin et al., 1992), the data shown in Figure 1 demonstrate a wide range of water supply conditions, from a level guaranteeing a good water supply in Kończewice in 1997 (420.7 mm) and 1999 (433.7 mm) to a severe deficit in Radzików in 2000, when only 143 mm rainfall was recorded during the whole vegetation period.



KOH - Kończewice, KOC - Pustków, RAH - Radzików and SMH - Smolice

Fig. 1. Seasonal and monthly sums of rainfall at various locations in 1997–2001

Due to these significant differences, an attempt was made to define the influence of seasonal rainfall sums on the baking qualities of wheat flour.

The correlation coefficients between the level of rainfall in the vegetation period and all the traits examined proved to be insignificant, suggesting that the rainfall had no influence on the technological value of the grain. This is contradicted, however, by the findings of other authors (e.g. Guttieri et al., 2001), who reported that moisture stress significantly affected traits such as grain weight, flour protein content, flour extraction qualities and grinding, as well as the physical and chemical properties of the dough. Similar results were obtained by Rharrabti et al. (2001), who found that, apart from yield, the protein content and SDS sedimentation were also affected by water and temperature stress.

It has long been known that the plant response to water deficit is strongly connected with the stage of development, yet opinions on this subject vary. Initially, the stages between flowering and maturity were regarded to be the most sensitive (Zagdańska and Pacanowska, 1979). Later on, this period was narrowed down to the phase of milky ripeness (Debacke et al., 1996). In more recent works, however, it seems that the influence of water stress on the yield was smaller when the stress occurred in the early vegetation phase than in a later stage of vegetation or after pollination (Abayomi and Wright, 1999). It can be assumed that, as in the case of other traits of practical importance, the influence of rainfall on technological parameters will depend on the distribution of the rainfall. In order to check this hypothesis, correlation coefficients were calculated between the monthly rainfall sums and the yearly average values of the traits. The results are presented in Table 1.

Table 1
Correlation coefficients between monthly rainfall sums and technological traits

Trait	Month				
	March	April	May	June	July
Sedimentation value	0.153	0.125	**–0.676****	–0.065	–0.138
Falling number	0.197	–0.286	0.465	–0.470	0.450
Protein content (%)	–0.103	–0.032	–0.200	0.032	–0.038
Water absorption.	0.326	–0.235	**–0.541***	–0.202	–0.033
Quality number	0.453	–0.007	–0.314	–0.234	0.025
Degree of softening	–0.403	0.453	–0.237	0.422	–0.256
Wet gluten (%)	–0.032	–0.028	–0.231	0.003	–0.012
Gluten index	0.039	–0.044	0.176	–0.156	0.069
Bread volume	0.201	0.168	–0.319	0.035	0.331
Estimated bread crumb	0.285	0.109	–0.328	–0.060	0.323
Grinding	–0.421	0.370	–0.422	0.450	–0.410

**, * - significant at P=0.01 and 0.05, respectively

The data presented in Table 1 indicated that in the period in question the correlation between rainfall levels and the average values of the traits varied. The correlation coefficients proved to be statistically insignificant, with the exception of the sedimentation value and water absorption in May. However, their variability over the period discussed could provide information about the determination of quality traits during the course of the vegetation period.

The highest values of the correlation coefficients were observed in May. The rainfall in that month significantly influenced the value of the sedimentation value (r= –0.676**) and water absorption (r= –0.541*), traits strongly connected with the gluten quality and rheological qualities of bread. The table indicates that in May the correlation coefficients were negative for almost all the technological traits (with the exception of the falling number and gluten index). It can therefore be assumed that too much rainfall in May has an unfavourable influence on the technological value of wheat grain. These results confirmed those reported by Kimball et al. (2001), indicating that drought slightly improves the grain quality. No data are available on this aspect under Polish conditions.

The lack of a significant correlation between the rainfall level and the majority of the technological parameters may result from the fact that the experiments involved genotypes which had not been tested before with regard to water deficit. It is thus possible that although they had different responses to this type of stress, these were masked when the results were averaged to calculate correlation coefficients. This phenomenon is also reported by Guttieri et al. (2001) and Rharrabti et al. (2001), who stated that the influence of water deficit on the value of some technological traits is also determined by the individual responses of the varieties. This is confirmed by the results presented in Table 2, which shows the average values, ranges and variability coefficients for traits correlated with the rainfall level in May. In order to facilitate the comparison,

the table also shows the rainfall sums in May at various locations in the experimental period. The highest variability for both of these traits was observed in Kończewice in 1998 and the lowest in Pustków in 2000, in spite of the fact that the level of rainfall was similar in both cases. In other cases, the variability coefficients of both traits had similar values despite large differences in rainfall (Kończewice 1997 and Smolice 1998). It can thus be assumed that variety traits had a decisive influence on the variability of sedimentation value and water absorption.

Table 2

Mean, range and coefficient of variability for sedimentation value and water absorption over years and locations

Year	Location	ΣR in May	Sedimentation value				Water absorption			
			Mean	Min.	Max.	CV%	Mean	Min.	Max.	CV%
1997	Kończewice	28.9	28.9	20.0	44.0	17.1	57.8	54.2	62.4	3.2
	Radzików	38.7	38.7	27.0	53.0	17.4	59.9	55.3	64.1	3.2
1998	Kończewice	61.4	61.4	35.0	93.0	21.3	61.4	53.6	68.2	4.6
	Radzików	86.0	86.0	68.0	95.0	6.3	63.6	56.2	67.3	3.2
	Smolice	78.1	78.1	47.0	95.0	15.4	62.0	55.8	66.8	3.7
	Pustków	81.6	81.6	58.0	98.0	10.9	64.4	59.3	67.5	3.0
1999	Radzików	74.6	74.6	59.0	87.0	9.6	60.2	55.6	64.0	3.0
	Kończewice	66.1	66.1	39.0	88.0	14.7	60.0	56.2	64.3	3.1
	Pustków	73.6	73.6	58.0	90.0	10.7	60.7	57.4	63.9	2.9
2000	Radzików	76.7	76.7	53.0	88.0	11.1	60.7	58.5	64.0	2.3
	Pustków	84.6	84.6	74.0	92.0	5.7	63.8	59.9	66.8	2.6
	Smolice	67.6	67.6	38.0	85.0	16.7	62.6	59.6	66.1	2.6
2001	Radzików	81.5	81.5	60.0	91.0	9.4	61.3	54.5	66.5	4.0
	Pustków	80.5	80.5	61.0	90.0	8.2	61.7	56.0	66.7	3.5
	$NIR_{0.05}$			**6.070492**				**0.912195**		

ΣR: Rainfall sum

Conclusions

1. The rainfall in May significantly influenced sedimentation value and water absorption, traits related to the gluten quality and rheological qualities of dough.
2. The factors determining the variability of sedimentation value and water absorption were variety traits, not rainfall.
3. The negative correlation coefficients for almost all technological traits (with the exception of falling number and gluten index) suggest that too much rainfall in May has an unfavourable influence on the technological value of wheat grain.

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BREEDING FOR RESISTANCE TO WHEAT NEMATODES (*HETERODERA AVENAE*)

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A new wheat variety resistant to cereal cyst nematode (CCN), CCNRV 1 (Raj Molya Rodhak 1), was developed from two genetically diverse cultivars in a single cross (J 24/AUS 15854). This variety exhibited a higher level of productivity in both CCN-infested and normal soils, with increases in the grain and straw yields of 78.7% and 60.1%, respectively, over Raj 3077 in infested soils. It also gave 19.0% higher yield than local varieties under timely-sown irrigated conditions in normal soils. It possesses superior grain quality along with other desirable agronomic traits. Genetically it carries a dominant gene for CCN resistance. It is a robust and reliable wheat variety that offers a high degree of resistance against nematodes in warmer areas of Rajasthan. It was recommended for timely-sown, irrigated conditions in CCN-infested areas of Rajasthan by the State Seed Sub-Committee on Crop Standards, Notification and Release of Varieties in September 2002. It is envisaged that this variety will help to boost wheat production and alleviate the socio-economic problems of subsistent Indian farmers in CCN-infested areas.

Key words: bread wheat, grain yield, dominant gene, molya disease, infested soils

Introduction

The problem of nematodes in wheat started to receive serious attention in India only from 1956 onwards, when a disease locally known in Rajasthan as 'molya', was found to be caused by a nematode parasite, *Heterodera avenae*. This disease is now known to occur in Rajasthan, Haryana, Punjab, Jammu & Kashmir, Himachal Pradesh and parts of Western Uttar Pradesh. More than 25 species of plant parasitic nematodes have been recorded on wheat in India; of these 6 are endoparasites and the rest belong to the category of ectoparasites. In spite of the fact that a large number of nematode species have been encountered in the rhizosphere of wheat plants, only two species, *Heterodera avenae* and *Anguina tritici,* are commonly important; four species, *Meloidogyne javanica*, *M. incognita*, *Pratylenchus thornei* and *Tylenchorhynchus vulgaris* are of potential importance, while the role of the rest of the species has not been investigated intensively so far. Apparently, molya disease is gradually spreading in two directions, (1) eastward towards the Indo-Gangetic Plains in Uttar Pradesh and (2) north-west towards Himachal Pradesh and Jammu & Kashmir. The greatest danger lies in the infestation of the Indo-Gangetic Plains, an important wheat belt for the country. Water rivulets and river water flowing through the infested areas, and sand or dust storms, are the main sources for the spread of molya disease in India.

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During the last 46 years or so, nematode disease has gradually spread, now covering more than one-third of southern Rajasthan, and has also spread to neighbouring states. The wheat grown in thirteen districts of Rajasthan is now prone to infection by molya disease initiated by *Heterodera avenae* every year. The total area of wheat cultivation in Rajasthan is about 2.05 million hectares (2001–02), of which about 0.15 million ha is mapped as affected by molya disease. The disease causes 40–50% yield losses, or even up to 60–65% in some cases (Mathur, 1969; Mathur et al., 1980). In terms of wheat production in the state (5.99 m tonnes), the CCN-infested area (0.15 m ha), yielded only 0.22 m tonnes in 2001–02 instead of 0.43 m tonnes, resulting in a monetary loss of about 126 million Rupees.

Of the many constraints preventing the realization of potential yield in wheat, the losses incurred due to molya disease are enormous. It has been observed that when high-yielding, well-adapted modern varieties are grown extensively in CCN-infested areas, the incidence of the disease reaches epidemic proportions and causes grain as well as straw yield losses. Earlier, efforts were made using cultural practices and chemicals to overcome the threat of CCN in naturally infested soils in Rajasthan, as the basis of genetically defined CCN resistance sources was not known at the time. It was felt that the deployment of CCN resistance resources could assist in achieving yield stability without resorting to potentially harmful chemicals, at the same time preventing environmental degradation and benefitting resource-poor farmers who could ill afford to use costly chemicals to sustain yields in infested soils (Mathur et al., 1998). Hence, breeding for CCN resistance based on genetic principles was initiated in 1991, soon after the identification of AUS 15854, a Turkish wheat line received from Australia, which demonstrated for the first time that resistance to CCN in wheat was controlled by a single dominant gene pair under warm conditions in Rajasthan, India. The progress achieved in checking the severe yield losses due to *Heterodera avenae* by developing a unique CCN-resistant wheat variety, CCNRV 1 (Raj Molya Rodhak 1), is dealt with in this paper.

Materials and methods

The screening of sources (varieties/lines) resistant to the cereal cyst nematode, *Heterodera avenae,* causing 'molya' disease in wheat was undertaken during the early nineties by nematologists at the Agricultural Research Station, Durgapura, Jaipur, Rajasthan, India. A large number of genotypes received from national and international sources such as CIMMYT Mexico, NBPGR, New Delhi and the Wheat Project Director of ARS, Durgapura, Jaipur and screened for CCN resistance under artificially CCN-infested field conditions. In addition, material obtained from other sources was also screened. A Turkish wheat line obtained from Australia (AUS 15854) was found to be resistant against local populations over the past 2–3 years in Rajasthan. It was also observed to show a very high degree of CCN resistance during testing at multi-locations (Delhi and Ludhiana) in 1989. Intensive testing was continued for a further year (1990) to confirm the resistance. As there was no decrease in grain yield or in other plant growth characters in comparison to the control, a uniform, CCN-resistant genotype (AUS 15854) was selected and included in a crossing block for further use in crossing programmes. Studies on the inheritance of

CCN resistance indicated that a single dominant gene was responsible for governing the inheritance of molya disease resistance in wheat line AUS 15854 (Mathur et al., 1994), making it amenable to improvement through breeding. This information was used to develop a new resistant variety, for the reliable production of wheat in CCN-infested areas.

A breeding programme for the development of a CCN-resistant wheat variety for the sustained maximization of wheat production in molya-infested areas of the country was initiated in 1991. Seven popular high-yielding bread wheat (*Triticum aestivum* L.) varieties, namely J-24, Raj 2184, Raj 3077, HD 2329, Raj 2535, HD 2009 and Kalyansona, were selected for the hybridization programme. The AUS 15854 genotype was used as the male parent in crosses with each variety to generate new CCN-resistant breeding material. This resulted in a heterozygous population of plants, which if large enough would include all the possible recombinants of the characters and genes of the parent plants. The pedigree method was followed in handling the segregating generations after hybridization. This pedigree method was based on the selection of single plants in an artificially CCN-infested field in each generation after crossing and growing them on as separate families of spaced CCN-infested plants. In the first instance selection was based on the visual assessment of single plants in the sick field, backed up by rapid CCN resistance and grain quality tests. Genetic variations were not fixed in these early generations, so the progeny of any single plant continued to vary to some extent in their agronomic traits. However, in subsequent generations, after selfing, the plants became more homozygous and true to type. Consequently, yield traits and CCN resistance were fixed in the fifth generation. A large proportion of the less promising families were rejected in each successive generation in all the seven crosses and a new variety is typically multiplied up from a single plant in the sixth generation. From each cross combination one of the best plant progenies was selected and bulked on the basis of CCN resistance and other desirable traits for further evaluation in yield trials at various locations. The seven selected plant progenies were designated as CCNRV 1 (J-24 × AUS 15854), CCNRV 2 (Raj 2184 × AUS 15854), CCNRV 3 (Raj 3077 × AUS 15854), CCNRV 4 (HD 2329 × AUS 15854), CCNRV 5 (Raj 2535 × AUS 15854), CCNRV 6 (HD 2009 × AUS 15854) and CCNRV 7 (Kalyansona × AUS 15854) for convenience in further testing.

Initially all seven selected CCN-resistant varieties were tested along with the check variety Raj 3077 in a randomized block design in three replications at twelve locations during the rabi season (46^{th} – 48^{th} week) of 1996–97. Three varieties, CCNRV 1, CCNRV 3 and CCNRV 7, were observed to have promising yield attributes combined with CCN resistance. These three varieties were tested in trials along with Raj 3077 during 1997–98 at six locations. Simultaneously all these varieties were also tested in demonstrations at KVK, Chommu, Jaipur and Rajasthan. Tests on the three CCN-resistant varieties and the check variety Raj 3077 were continued in 1998–99 at five locations in naturally CCN-infested fields. In both years the trials were conducted in a randomized block design with six replications. The plot size was 6.0 m × 5.52 m, accommodating 24 rows, 23 cm apart. Naturally CCN-infested fields with about 6–10 larvae/g soil were selected for each trial and demonstration. To assess the grain yield performance of CCNRV 1 in fields with normal soil (CCN-free soils), nine demonstrations were conducted in 2001–02, involving popular wheat varieties (Raj 3077, Raj 1482) and a local variety (Table 5). The crop was raised using 100 N : 40 P_2O_5 : 60 K_2O kg/ha fertilizers and recommended agronomic practices in both naturally infested and normal (nematode-free) soils. The grain and straw yield per plot were recorded in kilogrammes, and converted to q/ha for the interpretation of the results.

Agronomic characters, namely maturity (days), plant height (cm), number of tillers per plant, spike length (cm) and 1000-grain weight (g), were recorded. The number of cysts per plant and the nematode reaction were recorded every year in the demonstrations and experimental trials to confirm resistance against CCN. A standard scale, as suggested by Swaroop (1986), was used to record the nematode reaction in infested soils: resistant (0.0–4.0 cysts/plant), moderately resistant (4.1–9.0 cysts/plant), susceptible (9.1–20.0 cysts/plant) and highly susceptible (20.1 and above cysts/plant). The grain quality characters, namely hectolitre weight (kg), grain appearance, protein content (%) and sedimentation value (ml) of CCNRV 1 and Raj 3077 were analysed as suggested by Hanslas (1986). The data were analysed using the standard statistical methods suggested by Panse and Sukhatme (1967).

Results and discussion

The association of a nematode *Heteodera avenae* with 'molya' disease, which causes 40–50% yield losses (Mathur, 1969; Mathur et al., 1980) has been reported in sandy soils in India and the severity of this disease has been increasing every year in non-traditional areas, making it a major threat to sustainable wheat productivity in CCN-infested areas. On nematode-infested areas the germination of wheat seeds was delayed by 2–3 days. Affected fields usually appear patchy, with only one or a few patches in newly infested fields. The plants become stunted and have a pale, unhealthy appearance, with stiff, thin, narrow leaf blades. Tillering is greatly reduced, the culms being thinner and weaker. Diseased plants flower prematurely and ears, if formed, have very few grains. The root system of diseased plants shows a characteristic elongation of the main root with excessive branching at the tip, giving it a bunchy, twiggy appearance. A few roots may be present at the base of the plants, but the fibrous root system is almost negligible. Even these roots may show a typical branching pattern if invaded by infective larvae. Infected roots also show a slight swelling at the point of attachment of the nematode cyst. Such plants can be easily pulled out of the ground. In the case of continuous wheat cultivation, such patches gradually increase in extent and may cover the whole field during the course of 3–4 years. In badly infested fields there is practically no grain formation and such fields are not even worth harvesting (Swaroop, 1986).

In the field, disease symptoms become apparent about a month after sowing, becoming prominent by the end of January, the glistening white females ultimately turning into brown cysts by mid-March. In the next crop season the infective larval stage of the nematode is released into the soil intermittently from mid- or late October onwards from cysts left in the soil after the harvest of the last season's crop. At the time of wheat seed sowing, the soil temperature and moisture are very favourable for the accelerated emergence of larvae from the cysts, thus affecting the growth of the wheat plants and causing severe yield losses every year. This high rate of loss could be avoided by cultivating the CCN-resistant wheat variety Raj Molya Rodhak 1 in infested soils.

The pedigree of wheat variety CCNRV 1 (Raj Molya Rodhak 1) is J 24/AUS 15854. This variety is the first wheat variety resistant to cereal cyst nematode in the country and was developed by transferring a dominant resistance gene from the exotic germplasm AUS 15854 (Sharma and Sharma, 2000). The main characteristics of CCNRV1 are intermediate growth habit, light green foliage colour at the boot stage, waxy flag leaf (dorsal side), tapering spike shape, intermediate to compact spikes turning dusty white at maturity, medium awn length and awns shed before maturity. It matures in 115–120 days and possesses medium bold, amber-coloured, lustrous grains. The results obtained in the different experiments are presented below.

The results for CCNRV genotypes at twelve locations (1996–97) revealed that all seven CCN-resistant varieties significantly outyielded the Raj 3077 check variety in infested soils (Table 1) and also gave a significantly higher straw yield than the check. The maximum grain yield was recorded for CCNRV 1, followed by CCNRV 3 and CCNRV 7, and a similar trend was observed for the straw yield. All seven varieties showed resistance against nematodes, the number of cysts per plant ranging from 1.7–3.5. All the CCN-resistant varieties showed superiority for plant height and tillers per plant as compared with Raj 3077, but only three varieties, CCNRV 1, CCNRV 3 and CCNRV 7, were found to be significantly superior for spike length. These results indicated that among the seven newly developed CCN-resistant wheat varieties only three (CCNRV 1, CCNRV 3 and CCNRV 7) had excellent potential for grain and straw yield along with other desirable agronomic traits.

The results in Table 2 showed that all three CCN-resistant wheat varieties (CCNRV 1, CCNRV 3 and CCNRV 7) significantly outyielded the Raj 3077 check variety for grain yield and straw yield, and also showed superiority for plant height, spike length and tillers per plant. These varieties were observed to be resistant to CCN, the lowest number of cysts per plant being recorded for CCNRV 1, indicating the superiority of CCNRV 1 over all the other varieties.

Table 1

Performance of CCN-resistant wheat varieties and Raj 3077 (check) in multi-location trials in infested soils in 1996–97

Variety	Grain yield[+]	Straw yield[+]	No. of cysts/plant	Nematode reaction	Plant height	Spike length	Tillers /plant
CCNRV 1 (J-24 × AUS 15854)	39.2*	70.2*	1.7*	R[†]	82.3*	12.0*	5.6*
CCNRV 2 (Raj 2184 × AUS 15854)	32.0*	61.2*	3.5*	R	106.1*	8.1	5.1*
CCNRV 3 (Raj 3077 × AUS 15854)	38.6*	70.7*	2.5*	R	78.2*	13.0*	7.8*
CCNRV 4 (HD 2329 × AUS 15854)	32.0*	61.4*	3.1*	R	91.3*	8.1	5.6*
CCNRV 5 (Raj 2535 × AUS 15854)	30.3*	58.7*	3.0*	R	87.2*	8.3	5.0*
CCNRV 6 (HD 2209 × AUS 15854)	29.7*	58.9*	2.2*	R	92.8*	8.5	5.5*
CCNRV 7 (Kalyansona × AUS 15854)	38.5*	66.1*	3.4*	R	82.2*	11.5*	6.0*
Raj 3077	21.6	44.5	13.3	S[‡]	54.0	7.6	2.8
C.D. at 5%	1.8	2.6	0.7		2.4	1.4	1.5

[+]: q/ha; *Significant at the 5% level, [†] Resistant (0.0–4.0 cysts/plant), [‡] Susceptible (9.1–20.0 cysts/plant)

Table 2

Performance of CCN-resistant wheat varieties and Raj 3077 (check) in multi-location trials in infested soils in 1997–98

Variety	Grain yield (q/ha)	Straw yield (q/ha)	No. of cysts/plant	Nematode reaction	Plant height	Spike length	Tillers /plant
CCNRV 1	40.7*	72.4*	1.7*	R[†]	84.6*	6.0*	12.0*
CCNRV 3	39.8*	70.7*	2.3*	R	77.2*	7.1*	12.9*
CCNRV 7	38.5*	67.1*	3.2*	R	81.4*	6.0*	11.5*
Raj	24.1	44.4	13.0	S[‡]	55.7	2.4	7.5
C.D. at 5%	3.0	1.2	1.9		2.0	0.1	0.6

*Significant at the 5% level, [†] Resistant (0.0–4.0 cysts/plant), [‡] Susceptible (9.1–20.0 cysts/plant)

The results of demonstrations at Krishi Vigyan Kendra, Chommu, Jaipur (Table 3) revealed that the maximum percentage increase in grain and straw yield over the check variety Raj 3077 was recorded for CCNRV 1, followed by CCNRV 3 and CCNRV 7. In demonstrations all the varieties except the check variety showed a resistant reaction against nematodes. The results of multi-location trials (1998–99) revealed that all three CCN-resistant wheat varieties gave significantly higher grain and straw yields than Raj 3077 and also showed superiority for CCN resistance (Table 4). CCNRV 1 gave the highest grain and straw yield and the lowest number of cysts per plant among the varieties in the present study. These results confirmed that CCNRV 1 has good yield potential along with excellent resistance to nematodes; hence this promising wheat variety could offer an exciting opportunity for overcoming the stagnating yield plateau of wheat in infested soils in warmer areas of Rajasthan.

The results for the overall mean performance of CCNRV 1 revealed that this variety gave a 78.7% higher grain yield than Raj 3077 (Table 5). It also gave a 60.1% higher straw yield than the widely cultivated popular wheat variety Raj 3077 in CCN-infested soils at 23 locations. It was further seen that CCNRV 1 has excellent resistance against nematodes with an average number of cysts per plant of 1.5 cysts per plant over the three years compared with 14.0 cysts per plant for Raj 3077. The overall results of three years of experiments at 23 locations showed that the newly developed wheat variety has good yield potential and superior resistance against molya disease in sandy soils in Rajasthan. It is thus envisaged that this variety will help to boost wheat production and alleviate the socio-economic problems of subsistent Indian farmers in CCN-infested areas. To assess the grain yield potentiality of this variety in normal soils (free from nematodes), demonstration plots were planted at nine locations in Rajasthan, India in 2001–02.

Table 3
Performance of CCN-resistant wheat varieties and Raj 3077 (check) at Krishi Vigyan Kendra, Chommu, Jaipur in infested soils in 1997–98

Variety	Grain yield (q/ha)	Increase over check (%)	Straw yield (q/ha)	Increase over check (%)	Nematode reaction
CCNRV 1	40.3	75.2	73.5	62.2	R†
CCNRV 3	38.8	68.6	70.0	54.8	R
CCNRV 7	37.1	61.3	67.8	50.0	R
Raj 3077	23.0		45.2		S‡

† Resistant (0.0–4.0 cysts/plant), ‡ Susceptible (9.1–20.0 cysts/plant)

Table 4
Performance of CCN-resistant wheat varieties in multi-location trials in infested soils in 1998–99

Variety	Grain yield (q/ha)	Straw yield (q/ha)	No. of cysts/plant	Nematode reaction
CCNRV 1	43.9*	71.3*	1.2*	R†
CCNRV 3	41.4*	69.0*	2.2*	R
CCNRV 7	41.4*	65.0*	2.8*	R
Raj 3077	23.4	45.0	13.9	S‡
C.D. at 5%	0.9	1.4	2.3	

*Significant at the 5% level, †: Resistant (0.0–4.0 cysts/plant), ‡: Susceptible (9.1–20.0 cysts/plant)

Table 5
Mean performance of CCNRV 1 and Raj 3077 (C) varieties in multi-location trials in infested soils (1997–1999)

	1996–97		1997–98		1998–99		Mean	
	CCNRV1	Raj 3077	CCNRV1	Raj 3077	CCNRV1	Raj 3077	CCNRV1	Raj 3077
No. of locations	12		6		6			
Grain yield (q/ha)	39.2*	21.6	40.7*	24.1	43.9*	23.4	41.2	23.0
CD	1.8		3.0		0.9			
Increment[+] (%)	80.8		68.3		87.6		78.7	
Straw yield (q/ha)	70.2*	44.5	72.5*	44.0	71.3*	45.2	71.3	44.5
C.D.	2.6		1.2		1.4			
Increment (%)	57.8		64.7		57.7		60.1	
No. of cysts/plant	1.7*	13.3	1.7*	12.8	1.2*	15.9	1.5	14.0
C.D.	0.7		1.9		2.3			
Nematode reaction	R	S	R	S	R	S	R	S

[+]Increment: Increase over check

The results of the demonstrations indicated that the newly developed wheat variety has good adaptability and a high, stable grain yield under timely-sown, irrigated conditions in warmer areas of Rajasthan. The maximum yield recorded for this variety was 56.5 q/ha, with an average yield over nine locations of 47.6 q/ha (Table 6). The results further indicated that in normal soils the variety gave yields as good as those of widely cultivated wheat varieties (Raj 3077 and Raj 1482). It is noteworthy that this variety gave a grain yield 19.00% higher than that of local wheat varieties, suggesting that it could sustain and maximize the production and productivity of wheat in the state, particularly in CCN-infested soils.

An analysis of agronomic traits and grain quality revealed that this variety has excellent grain quality parameters as compared to Raj 3077 (Table 7). CCNRV 1 matures early and has good plant height and number of tillers, long spike length and excellent grain weight in infested soils. Therefore CCNRV 1, which carries resistance genes against nematodes and has good yield potential along with various other desirable parameters, was released for cultivation in Rajasthan state by the State Seed Sub-Committee on Crop Standards (Notification and Release of Varieties meeting held on 28th September, 2002). It is hoped that this wheat variety will help in achieving higher yield levels throughout the country in areas where molya disease is a threat to wheat cultivation.

Table 6
Performance of CCNRV 1 along with popular wheat varieties in multi-location trials in CCN-free soils in 2001–02

Location	Yield (q/ha)			
	CCNRV 1	Raj 3077	Raj 1482	Local
Durgapura (ARS)	54.1	55.2	45.0	40.2
Chommu (KVK)	42.2	44.3	–	–
Dausa (KVK)	36.0	37.2	38.0	33.0
Vansthali (KVK)	50.0	68.0	48.0	–
Tabiji –1 (ATC)	40.5	34.3	–	–
Tabiji –2 (ATC)	43.4	36.8	–	–
Chommu (FF)	56.5	59.7	53.7	46.5
Jaisingpura (FF)	50.5	53.0	51.2	39.0
Niwana (FF)	55.7	56.0	47.2	41.5
No. of locations	9	9	6	5
Mean	47.6	49.4	47.2	40.0

ARS =Agricultural research station, KVK = Krishi Vigyan Kendra, ATC = Adaptive trial centre, FF = Farmer's field.

Table 7
Mean performance of agronomic traits and grain quality parameters of CCNRV 1 and Raj 3077 (check) in infested soils

	CCNRV 1	Raj 3077
Agronomic traits		
Days to maturity	117.5	121.0
Plant height	83.4	54.8
Spike length	12.0	7.5
Tillers/plant	5.8	2.6
1000-grain weight	41.2	29.0
Grain quality parameters		
Hectolitre weight	76.0	54.5
Grain appearance	7.0	5.5
Protein percentage	12.7	11.8
Sedimentation value	55.0	58.0

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Short communication

EFFECT OF HERBICIDES ON THE WEED INFESTATION AND GRAIN YIELD OF SOYBEAN (*GLYCINE MAX*)

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Two field experiments were conducted during the *kharif* (rainy) season of 1999 and 2000 on a loamy sand soil to study the effect of various pre- and post-emergence herbicides on the weed infestation and grain yield of soybean. The presence of weeds in the weedy control plots resulted in 58.8 and 58.1% reduction in the grain yield in the two years compared to two hand weedings (HW) at 30 and 45 days after sowing (DAS), which gave grain yields of 1326 and 2029 kg ha^{-1}. None of the herbicides was significantly superior to the two hand weedings treatment in influencing the grain yield. However, the pre-emergence application of 0.75 kg ha^{-1} S-metolachlor, and 0.5 kg ha^{-1} pendimethalin (pre-emergence) + HW 30 DAS were at par or numerically superior to this treatment. There was a good negative correlation between the weed dry matter at harvest and the grain yield of soybean, which showed that effective weed control is necessary for obtaining higher yields of soybean.

Keywords: grain yield, hand weeding, herbicides, soybean, weeds

Introduction

Owing to sowing at wide row spacing (45 cm), initial slow growth and the rainy season, soybean (*Glycine max*) encounters severe competition from weeds, particularly in the early stages of crop growth. Since unchecked weed infestation causes considerable yield reductions, timely, effective weed management may increase the yield to a great extent. Two hand weedings are recommended for effective weed control in soybean (PAU, 1998). However, labour shortages, particularly during the critical crop-weed competition period, and inclement weather conditions lead to delayed weeding, which is ultimately costly and ineffective. Under such conditions, it was therefore deemed necessary to explore the possibility of developing an effective, feasible and acceptable weed management technology. Hence, two field experiments were conducted to evaluate the effects of different herbicides, applied pre-emergence or post-emergence, on the weed infestation and grain yield of soybean.

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Materials and methods

Two field experiments were conducted during the *kharif* (rainy) season of 1999 and 2000 at the Punjab Agricultural University, Ludhiana, India, to test the performance of different herbicides on the weed infestation and grain yield of soybean. Meteorological data pertaining to the crop seasons of the two years are given in Table 1. In both the experiments 11 treatments, as given in Tables 2 and 3, were compared in a randomized complete block design. Variety SL 295 was sown in the first week of June in rows 45 cm apart using a 75 kg ha^{-1} seed rate. A fertilizer dose of 30 kg N and 60 kg P_2O_5 ha^{-1} was applied at the time of sowing. Pre-emergence herbicides were applied immediately after sowing and post-emergence herbicides were sprayed 18 days after sowing. All the herbicides were sprayed with a knapsack sprayer using 500 litres of water per hectare. Data on the dry weight of weeds were recorded at crop maturity. Weed control efficiency was calculated as follows:

100 × (Dry weight of weeds in weedy control plot – Dry weight of weeds in treated plot)/ Dry weight of weeds in weedy control.

Information on the weed survey was also collected and has been given in the Results section.

Table 1
Meteorological data during the crop season (1999 and 2000)

Month	Temp. (°C)		Relative humidity (%)	Rainfall (mm)
	Min	Max		
1999				
June	24.6	36.9	55	24.4
July	26.7	33.9	71	359.2
August	25.5	33.8	77	68.6
September	24.7	34.1	74	92.6
October	17.5	33.1	64	0.0
2000				
June	27.1	36.1	62	88.2
July	26.8	33.2	80	189.4
August	26.0	34.1	79	120.8
September	22.7	34.0	73	139.2
October	18.5	33.8	62	0.0

Results

The dominant weed flora of the experimental sites comprised *Cyperus rotundus*, *Cynodon dactylon*, *Dactyloctenum aegyptiacum*, *Commelina benghalensis*, *Eragrostis pilosa*, *Digera arvensis*, *Euphorbia hirta*, *Tribulus terrestris*, *Leucus aspera* and *Celosia argentea*. It was further observed that the grassy weed flora out-numbered the broad-leaved weeds. *Cyperus rotundus*, *Dactyloctenum aegyptiacum* and *Commelina benghalensis* constituted the major population.

Pendimethalin 0.5 kg ha^{-1} + HW 30 DAS had the highest weed control efficiency (WCE) during both the years (Table 2), closely followed by 2 HW done 30 + 45 DAS. On the basis of the means of the two-year data the other treatments having high WCE were the pre-emergence application of 0.75 kg ha^{-1} S-metolachlor and the post-emergence application of 37.5 or 50.0 g ha^{-1} quizalofop-ethyl. Imazamox had the lowest WCE.

The pre-emergence application of 0.75 kg ha^{-1} S-metolachlor resulted in the highest grain yield of soybean in both the years (Table 3), but this was statistically at par with 2 HW done 30 + 45 DAS and with the pre-emergence application of 0.5 kg ha^{-1} pendimethalin + HW 30 DAS. The other promising treatments were alachlor and imazamox + imazethapyr. Of all the herbicides, the post-emergence application of 40 g ha^{-1} imazamox gave the lowest grain yield. The pre-emergence herbicides, in general, performed better than the post-emergence herbicides. Compared to 2 HW 30 + 45 DAS, the weedy control decreased the grain yield by 58.8 and 58.1% in 1999 and 2000 years, respectively. One HW 45 DAS led to a 946 kg ha^{-1} grain yield compared to 1326 kg ha^{-1} with 2 HW 30 + 45 DAS, thus involving a 28.6% reduction in yield.

Table 2
Effect of various weed control treatments on weed control efficiency in soybean

Treatment	Herbicide application rate*	Herbicide application time	Weed control efficiency (%)		
			1999	2000	Mean
Alachlor	2.00 kg	Pre-emergence	40.2	50.4	45.3
S-Metolachlor	0.50 kg	Pre-emergence	15.6	55.8	35.7
S-Metolachlor	0.75 kg	Pre-emergence	32.8	71.5	52.1
Pendimethalin+HW 30 DAS	0.50 kg	Pre-emergence	49.1	90.1	69.6
Quizalofop-p-ethyl	37.5 g	Post-emergence	42.6	57.3	49.9
Quizalofop-p-ethyl	50.0 g	Post-emergence	46.7	52.4	49.5
Imazamox	40.0 g	Post-emergence	4.9	8.3	6.6
Imazamox + Imazethapyr	75.0 g	Post-emergence	30.3	51.9	41.1
Quizalofop-p-tefuryl	50.0 g	Post-emergence	–	62.7	–
1 HW 45 DAS			40.2	–	–
2 HW 30 + 45 DAS			46.7	89.2	67.9

*: (ai ha^{-1}); – : not tested

Table 3
Effect of various weed control treatments on the grain yield of soybean

Treatment	Herbicide application rate*	Herbicide application time	Grain yield (kg ha^{-1})		
			1999	2000	Mean
Alachlor	2.0 kg	Pre-emergence	1242	1818	1530
S-Metolachlor	0.5 kg	Pre-emergence	1067	1852	1460
S-Metolachlor	0.75 kg	Pre-emergence	1465	2348	1907
Pendimethalin+HW 30 DAS	0.5 kg	Pre-emergence	1360	1995	1678
Quizalofop-p-ethyl	37.5 g	Post-emergence	1168	1751	1460
Quizalofop-p-ethyl	50.0 g	Post-emergence	1158	1507	1333
Imazamox	40.0 g	Post-emergence	633	909	771
Imazamox + Imazethapyr	75.0 g	Post-emergence	1353	1776	1565
Quizalofop-p-tefuryl	50.0 g	Post-emergence	–	1801	–
1 HW 45 DAS			946	–	–
2 HW 30 + 45 DAS			1326	2029	1678
Weedy control			545	850	698
LSD (P=0.05)			246	405	

*: (ai ha^{-1}); – : not tested

Discussion

The high yields obtained with some of the treatments (Table 3) were the result of effective weed control, as can be seen from the weed control efficiency (Table 2). The treatments giving low yields had poor weed control efficiency. In soybean, high weed control efficiency and consequently high grain yield have been reported with the use of pendimethalin (Jain et al., 2000; Nayak et al., 2000) and two hand weedings (Jain et al., 2000; Mandloi et al., 2000). Further, compared to the sole application of herbicides, the integration of pre-emergence herbicides with one HW resulted in lower weed dry matter and higher grain yield of soybean (Balyan et al., 1999). One HW 45 DAS gave a 28.6% reduction in the grain yield compared with 2 HW 30 + 45 DAS. This shows that one HW 45 DAS was not sufficient to control weeds effectively and to obtain high yields, as the presence of weeds caused considerable losses. A good negative correlation was observed between the weed dry matter at harvest and the grain yield of soybean (Fig. 1), which clearly shows that effective weed management is a pre-requisite for obtaining higher yields of soybean.

In all the treatments WCE was lower in 1999 than in 2000, possibly due to differences in meteorological parameters such as rainfall and relative humidity (Table 1). In 2000 higher rainfall and relative humidity (in June and July) were recorded than in 1999, which resulted in higher weed biomass; the weed biomass in the weedy control plots amounted to 2738 and 4579 kg ha^{-1} in 1999 and 2000, respectively.

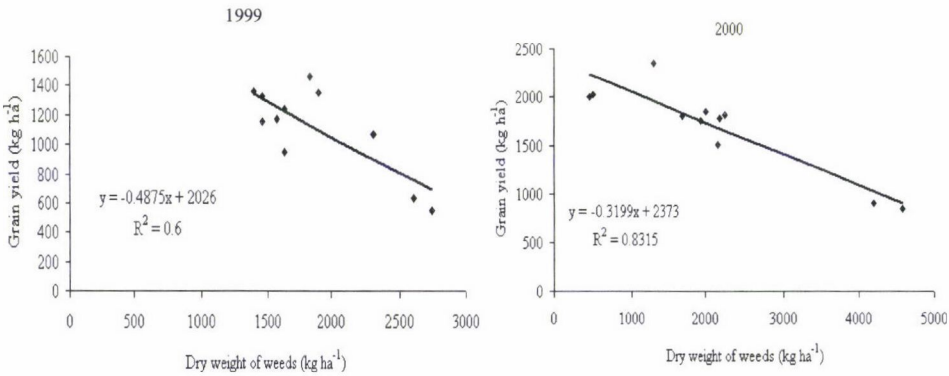

Fig. 1. Relationship between dry weight of weeds and grain yield of soybean (1999 and 2000)

Conclusions

Weed control treatment involving two hand weedings at 30 and 45 DAS is effective in obtaining high yields of soybean. Among the herbicides, the pre-emergence application of S-metolachlor at 0.75 kg ha^{-1} alone or pendimethalin at 0.5 kg ha^{-1} integrated with 1 HW 30 DAS provided effective weed control and high yields of soybean.

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Short communication

RELATIVE EFFICIENCY OF ANDROGENESIS AND MAIZE-MEDIATED PRODUCTION FREQUENCIES OF POLYHAPLOIDS IN WINTER × SPRING WHEAT AND TRITICALE × WHEAT HYBRIDS

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Comparisons between androgenesis and maize-mediated haploid production efficiencies were made in six F_1 genotypes each of winter × spring wheat and triticale × wheat crosses. The haploid status of the plantlets obtained was confirmed through cytological examination of the root tips. Much higher embryo formation (15.2%), haploid induction (8.7%) and doubled haploid production (8.3%) were obtained in the winter × spring wheat F_1s through the wheat × maize system than by androgenesis (3.1%, 3.2 and 2.7%, respectively). Three of the triticale × wheat F_1 genotypes failed to respond to androgenesis, while no haploids were recovered through the wheat × maize system in any of the six triticale × wheat F_1s. Genotypic specificity, low callus induction and albinism reduced the efficiency of androgenesis both in winter × spring wheat and triticale × wheat hybrids. In all, the wheat × maize system proved to be better for winter × spring wheat hybrids and androgenesis for triticale × wheat hybrids.

Key words: androgenesis, doubled haploid, genotypic specificity, triticale × wheat hybrids, wheat × maize system

Introduction

The winter ecotypes of wheat (*Triticum aestivum*) and triticale (× *Triticosecale*) constitute reservoirs of useful genes for the genetic enrichment of spring wheat. The success of winter × spring wheat and triticale × wheat breeding programmes depends upon the isolation of promising homozygous recombinants. Doubled haploid breeding allows the development of completely homozygous lines from the F_1 generation in a single step. However, the efficiency of a haploid production technique should be high, so that many doubled haploid lines are generated for effective selection. Kisana et al. (1993) and Sadasivaiah et al. (1999) observed that genotypes which did not respond to anther culture might respond in wheat × maize crosses. Gury et al. (1993) indicated that haploid calli induction and regeneration through androgenesis were lower compared with the wheat × maize system. The present study was undertaken to investigate the relative efficiency of the two haploid production techniques in winter × spring wheat and triticale × wheat hybrids and to determine their feasibility.

Materials and methods

Six diverse genotypes each of winter wheat and triticale were crossed with six spring wheats in pairs to produce the 12 F_1 combinations listed in Table 1. The F_1s were raised in the field and twenty plants of each F_1 genotype were subjected to anther culture as well as wide hybridization with maize. The relative efficiency of androgenesis and the wheat × maize system was evaluated based on the efficiency of calli induction/embryo formation, haploid plantlet regeneration and doubled haploid production. The genotypic specificity and practical feasibility of the techniques were also determined and cases of albinism were recorded.

For anther culture, 30–39 anthers per spike (about 720 anthers per F_1 hybrid) with pollen at the mid- to late uninucleate stage were subjected to chilling treatment at 4°C for 48 h prior to culture. The anthers were cultured in Potato II medium (Chuang et al., 1978) with modifications. The medium was modified by replacing sucrose with maltose (90 g l^{-1}) as suggested by Last and Brettell (1990) and 1 g l^{-1} glutamine, 0.5 mg l^{-1} kinetin and 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid were added to the medium for callus induction, after which 20 ml medium was poured into test tubes and autoclaved. The anthers were cultured in test tubes under sterile laminar air flow conditions. The anther cultures were initially incubated for 8 days in the dark at a temperature of 28±1°C in a growth chamber and then shifted to a temperature of 20±1°C with an alternating photoperiod of 10 h light/14 h dark in the growth room. The calli obtained were subcultured to regeneration medium (Chuang et al., 1978). The regenerants were transferred to liquid rooting medium (½ strength MS salts, no sucrose and 1 mg l^{-1} each of naphthaleneacetic acid and indole-3-butyric acid) and later transferred to soil in small pots. Cytological examination of the root tips was done to ascertain the haploid status of the plantlets. The haploids were treated with colchicine at the three- to five-tiller stage (Inagaki, 1985) and transferred to soil in larger pots. The doubled haploid seeds were harvested from the plants upon maturity.

For intergeneric hybridization with maize, 20 spikes of each F_1 genotype were emasculated before anthesis and 30–35 florets per spike (about 640 florets per F_1 hybrid) were pollinated with pollen from the maize cultivar Early Composite. Twenty-four hours after pollination, the uppermost internodes of spikes pollinated with maize pollen were injected with 2 ml of 2,4-D solution (100 mg l^{-1} concentration), using a syringe with a fine needle, after which the hole was sealed with paraffin wax. The injections were repeated on two more consecutive days. The seeds formed through wheat × maize hybridization were harvested 18 days after pollination and those with an embryo were identified following the procedure of Bains et al. (1998). The embryos were cultured in test tubes containing 20 ml of MS medium supplemented with 0.5 mg l^{-1} kinetin, 400 mg l^{-1} L-glutamine, 20 mg l^{-1} each of L-arginine, L-cysteine and L-leusine, 30 g l^{-1} sucrose and 8 g l^{-1} agar-agar. The embryo cultures were incubated in the dark at 20±1°C in the growth room until regeneration was initiated. Shoots and roots developed after about one week of culture. The subsequent procedure for the production of doubled haploids was similar to that followed for anther culture.

Observations were recorded on calli/embryos induced, green plantlet regeneration, albinism and doubled haploid production. The differences between the two haploid production systems were analysed using Fisher's 't' test.

Results and discussion

Cytological examination of the regenerated plantlets revealed a set of 21 chromosomes, thus confirming their haploid status. The relative efficiency of embryo formation through wheat × maize hybridization was significantly greater (based on the 't' test) than that of callus induction through androgenesis in winter × spring wheat hybrids (Table 1). One of the wheat F_1 genotypes failed to

respond to androgenesis, suggesting genotypic specificity, which limits its extensive use in wheat haploid production. The wheat × maize system, however, was genotype non-specific. Sadasivaiah et al. (1999) suggested that reduced genotype specificity made the wheat × maize system more efficient than anther culture for the production of haploids in wheat.

The maize-mediated system was ineffective for the production of haploid embryos in triticale × wheat hybrids. Similar results were obtained by Inagaki et al. (1997) in crosses of hexaploid triticales and substituted triticales with maize, in which a negligible frequency of embryos was obtained. However, haploid calli were obtained from triticale × wheat hybrids through androgenesis. Therefore, androgenesis may be effective for fixing introgressed rye genes into a wheat genetic background (Tao and Hu, 1989).

A significantly higher percentage of haploid regeneration and doubled haploid production was obtained through the wheat × maize system than by androgenesis in winter × spring wheat hybrids (Table 1), and this can be attributed to the well-developed haploid embryos, having both root and shoot primordia, formed in the wheat × maize system. Redha et al. (2000) reported that the early transfer of anthers to regeneration medium after 28 days on induction medium brought about a reduction in the number of embryos formed, but nevertheless significantly improved their quality and the frequency of plant regeneration. This anther transfer technique may improve the efficiency of the anther culture response in wheat. The studies of Gury et al. (1993) and Kisana et al. (1993) support the finding that regeneration was better after intergeneric hybridization.

Androgenesis displayed genotypic specificity both in winter × spring wheat and triticale × wheat hybrids, and not all the crosses were responsive to anther culture. High frequencies of albino plantlets were obtained in winter × spring wheat and triticale × wheat hybrids after androgenesis. No albinos were obtained through the maize-mediated system in winter × spring wheat hybrids.

Overall, the relative efficiency of the wheat × maize system with respect to haploid induction in winter × spring wheat hybrids is greater than that of androgenesis due to the low callus induction response, genotypic specificity and albinism. Therefore, the present study supports the findings of Sadasivaiah et al. (1999), Gury et al. (1993) and Kisana et al. (1993), that the wheat × maize system is preferable for haploid production in wheat. Singh et al. (2001) and Sharma et al. (2002) also assessed the wheat × maize system and suggested its use for the production of doubled haploids in wheat.

In triticale × wheat hybrids, on the other hand, androgenesis is a viable haploid production technique. Nevertheless, further studies are required to improve the efficiency of the maize-mediated system of haploid induction in triticale × wheat hybrids by studying the influence of maize genotypes and the use of different plant growth regulators to enhance haploid induction responsiveness.

Table 1

Relative efficiency of androgenesis and wheat × maize systems for the production of haploids and doubled haploids in winter × spring wheat and triticale × wheat hybrids

No.	F_1 Genotypes	Androgenesis				
		ac	ci	a	gp	dh
Winter × spring wheat F_1s						
1.	Bounty × VL784	725	0	–	–	–
2.	Envoy × VL763	727	32 (4.4)	3 (9.37)	2 (6.25)	1 (3.12)
3.	Pnfjoumee × HPW93	727	24 (3.3)	0	1 (4.16)	1 (4.16)
4.	Saptdhara × HPW147	722	26 (3.6)	5 (19.23)	1 (3.84)	1 (3.84)
5.	Sentry ×RL 10–22	733	11 (1.5)	1 (9.09)	0	–
6.	WW24 × HPW89	722	39 (5.4)	1 (2.56)	2 (5.12)	2 (5.12)
	Mean (%)		3.1	6.7	3.2	2.7
Triticale × wheat F_1s						
1.	DT91 × VL763	750	15 (2.08)	3 (20.00)	1 (6.66)	1 (6.66)
2.	DT103 × HPW89	709	0	–	–	–
3.	DT109 × HPW93	715	0	–	–	–
4.	TL2900 × HPW147	777	7 (0.97)	1 (14.28)	0	–
5.	TL2901 × RL10–22	736	14 (1.94)	0	1 (7.14)	1 (7.14)
6.	TL2908 × VL784	717	0	–	–	–
	Mean (%)		0.8	5.71	2.30	2.30
No.	F_1 Genotypes	Wheat × maize system				
		fp	sf	ef	r	dh
Winter × spring wheat F_1s						
1.	Bounty × VL784	638	370 (57.9)	63 (17.02)	5 (7.93)	5 (7.93)
2.	Envoy × VL763	641	408 (63.6)	66 (16.17)	7 (10.60)	6 (9.09)
3.	Pnfjoumee × HPW93	638	338 (52.9)	45 (13.31)	3 (6.66)	3 (6.66)
4.	Saptdhara × HPW147	643	465 (72.3)	91 (19.56)	8 (8.79)	7 (7.69)
5.	Sentry ×RL 10–22	639	329 (51.4)	46 (13.98)	3 (6.52)	3 (6.52)
6.	WW24 × HPW89	642	312 (48.6)	34 (10.89)	4 (11.76)	4 (11.76)
	Mean (%)		57.9	15.15	8.71	8.27
Triticale × wheat F_1s						
1.	DT91 × VL763	633	0	–	–	–
2.	DT103 × HPW89	641	0	–	–	–
3.	DT109 × HPW93	632	0	–	–	–
4.	TL2900 × HPW147	639	0	–	–	–
5.	TL2901 × RL10–22	636	0	–	–	–
6.	TL2908 × VL784	643	0	–	–	–

ac = anthers cultured; ci = calli induced; a = albinism; gp = green plantlets; dh = doubled haploids; fp = florets pollinated; sf = seed formation; ef = embryo formation; r = regeneration; % in parentheses

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EFFECT OF THE RIDGE TILLAGE SYSTEM ON SOME SELECTED SOIL PHYSICAL PROPERTIES IN A MAIZE MONOCULTURE

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Within the framework of cooperation between Szent István University and the Vienna University of Agricultural Sciences, a soil cultivation experiment in a maize (*Zea mays* L.) monoculture was set up for the first time in Austria near Pyhra (Lower Austria) in 1996. A study was conducted to evaluate the effects of ridge tillage (RT) in comparison with conventional mouldboard ploughing in autumn (CT) and no-tillage (NT) on the penetration resistance (PR), soil bulk density (BD) and porosity (P) of sandy loam soil (Typic Agriudoll). Analyses were made for each treatment and for different parts of the ridge (top and side of the ridge, and interrow) in 1998, 2000 and 2002. The average PR and BD values were greatest in the no-tillage plot, being 3.42 MPa and 1.56 $g \cdot cm^{-3}$, respectively. After six years, ridge tillage resulted in lower penetration resistance and bulk density values in the upper 20 cm than conventional tillage and no-tillage. Ridge tillage appears capable of reducing compaction in this soil. It can be concluded from the results that ridge tillage is capable of maintaining and improving favourable physical conditions in the soil.

Key words: conventional tillage, no-tillage, ridge tillage, maize, penetration resistance, bulk density, porosity

Introduction

The use of conventional tillage systems based on mouldboard ploughs on poorly drained soils has resulted in a gradual deterioration of the soil structure. Conservation tillage, which involves the maintenance of crop residues on the soil surface, effectively controls soil erosion (Harrold and Edwards, 1974; Langdale et al., 1979) and enhances soil water conservation (Buchele et al., 1955; Mielke et al., 1986; Unger, 1984). According to the Agricultural Research Service (1981) conservation tillage means any tillage and planting system that leaves at least 30% of the soil surface covered by residues after planting. Conservation tillage is an "umbrella" term encompassing several tillage systems including minimum tillage, reduced tillage, no-tillage, mulch tillage and ridge tillage (Mannering and Fenster, 1983). However, the adaptation of conservation tillage for maize (*Zea mays* L.) production on poorly drained soils is limited because it often results in lower yields than for maize grown under conventional tillage (Dick and Van Doren, 1985; Griffith et al., 1973). No additional tillage occurs until the planting of the succeeding crop. The crop is planted into the top of the ridge, usually following ridge truncation. One or two interrow cultivations are used to control weeds and reform the ridges for the next growing season.

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Ridge tillage is a row-crop production system with the aim of soil protection and cultivation. Ridge tillage is characterized by a permanent row-interrow configuration where the row is elevated 12 to 20 or 22 cm above the interrow throughout most of the year (Stone et al., 1989; Lal, 1990; Liebig et al., 1993; Birkás et al., 1998). The soil is left undisturbed from harvest to planting except for strips up to 1/3 of the row width. Planting is done on the ridge and usually involves the removal of the top of the ridge. Planting is carried out using sweeps, disk openers, coulters or row cleaners. Residue is left on the surface between the ridges. Weed control is accomplished with crop protection products (frequently banded) and/or cultivation. The ridges are rebuilt during row cultivation. The method has been known for centuries in the United States and in certain countries of Africa and is included in soil conservation systems (Lal, 1990; Vyn et al., 1990). The method has no precedence in Hungarian literature, and the same is true for the whole Central European region. The first publication on this topic was written by Birkás et al. (1998) in Hungary, and reported the results of soil condition analysis and yield in a ridge tillage trial set up in Gödöllő in 1995.

Tillage decreases soil mechanical resistance as measured by the cone index (Cox et al., 1990), but this is accompanied by a decrease in macropore continuity, as earthworms produce fewer biochannels than under reduced tillage conditions (Gantzer and Blake, 1978). The use of a no-tillage cropping system achieves the greatest surface residue retention. However, there is concern regarding the potential for the development of unfavourable soil physical conditions due to traffic and because the soil is not loosened by tillage. Compaction due to equipment traffic is usually of the greatest concern. However, surface and subsurface soil density and penetration resistance may increase naturally when using a no-tillage system (Ehlers et al., 1983; Mielke et al., 1986). Increased soil bulk density and penetration resistance may reduce water infiltration, plant root growth and crop yields. These consequences result from raindrop impact and the structural failure (collapse) of soils having low-stability aggregates. Soils with a high sand content are especially prone to develop a dense zone with high penetration resistance. With no-tillage, natural increases in soil bulk density and penetration resistance are usually limited to the upper 15 cm of the soil profile (Unger, 1996).

It has also been shown that ridge tillage systems can achieve yields equivalent to mouldboard-plough-based tillage systems. Moreover, the stubble remaining in the spacing reduces treading damage in traffic furrows (Hayes, 1982; Liebig et al., 1993) and the evaporation of soil moisture (Unger, 1995). Farmers often use matched-width equipment in ridge tillage so that tractor wheel traffic for herbicide and fertilizer application, planting and ridging is confined to the same interrows. As a result, three distinct soil environments exist in ridge tillage: trafficked interrows, non-trafficked interrows and rows.

In Austria about 2.3 million ha is affected by water erosion, while 380,000 ha are considered to be moderately to highly erosive. The yearly loss of soil is estimated to be 8 million tons. Radke (1982) concluded that ridge tillage could help control erosion by leaving crop residues on the surface. Erosion and nutrient losses with ridge tillage were 67% less than in conventional tillage on land with 8 to 12% slope (Römkens et al., 1973).

Buhler (1995), Clements et al. (1996), Gail et al. (1996) and Klein et al. (1996) emphasized the low weed coverage, especially in the spacing, while on the ridges the threat of weeds increases because of the quicker warming of the soil and the better loosening.

Dickey and Jasa (1987) and Hayes (1982) reported several farms in the USA where ridge tillage was extended to the whole area after seeing the advantages of this system. The number of crops that can be cultivated by this method is relatively low (maize, soy bean, sorghum, sometimes sugar beet), but crop rotation is possible (Klein et al., 1996). The ridge tillage method shows favourable results from the economic point of view as compared with conventional ploughing systems. Hayes (1982) claimed that the work demand, and the fuel and machine costs were lower (although it requires special machinery), and the operations can be timed well. Weersink et al. (1992) compared the input-output ratio of different tillage systems on the basis of models. When comparing the ploughing, cultivator, direct drilling and ridge tillage systems the greatest net income was produced by ridge tillage on both clay and sand soils.

When analysing ridge tillage the main objective for all authors (Benjamin et al., 1990; Vyn et al., 1990; McInnes et al., 1991; Stonehouse, 1997) is not an increase in yield but the conservation of the soil, and therefore this method is recommended mostly on sloping fields requiring protection, where in this way higher yield can also be guaranteed.

Materials and methods

This experiment was set up in Pyhra (St. Pölten), located about 80 km west of Vienna (Lower Austria) on a < 0.1% slope in the autumn of 1996. This region is found in the foothills of the Alps, 325 m above sea level. The landscape is characterised by gentle to fairly steep slopes. The average long-term annual rainfall amount is 725 mm (1901–1990), and the mean annual temperature is 8.8°C. The soil was classified as sandy loam Typic Agriudoll (USDA SCS, 1992) which typically has a mixed mineralogy and mesic temperature regime. The average soil texture analysis of this soil is 34% sand, 46% silt and 20% clay in the surface horizon (0–20 cm depth) and an average organic carbon content of about 1.3%.

The study was conducted on the experimental field of the Pyhra Agricultural School on small plots where maize (*DK 210* – 1997, 1998; *Magister RFZ 290* – 1999; *Coach* – 2000, 2001; *Brissac* – 2002) was grown in a monoculture. The experimental design was a one-factor, small plot trial arranged in strips. The plot size was 9 m × 50 m (450 m^2). Three tillage systems were compared in the experiment, no-tillage, conventional tillage with mouldboard ploughing (to 15 cm) in autumn and ridge tillage. The number of replications (r) was 4, and the treatments were randomised over the area.

At the beginning the soil was prepared for no-tillage in one process at the same time as sowing and consisted only of the loosening of the seedbed. In the case of conventional tillage the medium deep ploughing (20–25 cm) in autumn and the smoothing were carried out in one process. Ridge tillage consists of planting on existing ridges and rebuilding the ridge during cultivation. The autumn soil preparation in the case of ridge tillage was the same in the year 1996 as in the case of conventional tillage, and ridges were formed in spring. A potato ridge-filling cultivator was used to form the initial 18–20 cm high ridges with a spacing of 70 cm and to cultivate and rebuild the ridges. In June the ridges were readjusted with the cultivator, and this also served as mechanical weed killing. In 1997 and 1998 there was no basic tillage in autumn, and the next year ridges were formed before sowing and after sowing in June. From 1999 on, autumn ploughing was also carried out in the ridge tillage treatment because of the increased weed infestation.

All the treatments were planted with a seed drill at the same seeding rate and depth, as early as the soil and weather conditions permitted. Fertilizer was applied equally to all treatments. Chemical pest and disease control was not needed because of the low occurrence. Herbicides were applied only in the no-tillage treatment (twice, in May and June) and in the conventional tillage treatment (once in June). In 1997 and 1998 no chemical treatment was applied in the ridge tillage treatment, but after that period herbicide was applied as in the other two treatments.

The evaluation of the physical characteristics of the soil involved measurements on the penetration resistance, bulk density, moisture content (measured with an electronic penetrometer from Szarvas) and porosity (calculated from the pF chart). Penetration resistance was measured with an electronic penetration probe at five depths (10, 20, 30, 40, 50 cm). Data were collected in three positions on the ridge: at the top and side of the ridge, and in interrows not affected by tractor wheel tracks. Bulk density was determined from soil cores (0.053 m by 0.05 m height) taken from interrows at a depth of 5–10 cm and 15–20 cm in each plot. Penetration resistance and bulk density values were used for statistical analysis. The effect of the treatments was analysed using one-way analysis of variance.

Results

The penetration resistance values measured in the upper 50 cm soil layer are presented in Table 1. Two years after the setting up of the experiment (1998) no statistically significant difference was detected in the uppermost layer. In the 10–20 cm layer PR exceeded 1.4 MPa only in the no-tillage treatment and in the interrow area of ridge tillage, but for the given soil conditions and moisture content even this value is not indicative of harmful compaction.

During the analysis carried out in 2000 the effect of the lack of soil tillage was most evident in the uppermost soil layers (0–10 and 10–20 cm) in no-tillage. The highest penetration resistance was measured in no-tillage, which exceeded the critical value of 2.5 MPa, while below 20 cm the penetration resistance was 2.5–3.5 MPa in all plots. In the sixth year of the experiment (2002) the soil resistance values equalized in the upper soil layer and did not exceed 2.00 MPa in any of the treatments. No compacted plough layer was evident, but it is interesting that the penetration resistance did not increase to a statistically significant degree in no-tillage, compared to the ploughing treatments.

Table 1
Effect of tillage treatments on penetration resistance [MPa]
Soil moisture content (mass % average of 0–50 cm): 1998: 20.4%; 2000: 18.3%; 2002: 15.6%

Soil depth (m)	Soil cultivation systems				
	No-tillage	Conventional tillage	Ridge		
			Top	Side	Interrow
October 1998					
0.00–0.10	1.37[a]	0.98[a]	0.90[a]	1.13[a]	1.13[a]
0.10–0.20	1.46[b]	1.13[a]	1.13[a]	1.03[a]	1.83[c]
0.20–0.30	1.51[a]	1.53[a]	1.47[a]	1.86[a]	2.17[a]
0.30–0.40	1.63[a]	1.59[a]	2.27[b]	2.06[b]	1.70[a]
0.40–0.50	1.60[a]	1.55[a]	2.11[a]	1.87[a]	1.65[a]
August 2000					
0.00–0.10	3.42[c]	1.10[a]	1.50[a]	1.57[ab]	1.97[b]
0.10–0.20	2.50[b]	1.12[a]	2.50[b]	2.25[b]	2.10[b]
0.20–0.30	2.62[a]	2.50[a]	2.50[a]	2.50[a]	2.77[a]
0.30–0.40	2.60[a]	2.27[a]	2.62[a]	2.67[a]	3.10[a]
0.40–0.50	2.65[a]	2.07[a]	2.60[a]	2.67[a]	3.35[a]
May 2002					
0.00–0.10	1.87[a]	1.45[a]	1.45[a]	1.25[a]	1.45[a]
0.10–0.20	1.92[bc]	1.52[ab]	1.37[a]	1.90[bc]	2.30[c]
0.20–0.30	1.57[a]	2.25[a]	2.47[a]	2.20[a]	2.30[a]
0.30–0.40	1.22[a]	2.40[c]	2.00[bc]	1.62[ab]	1.55[ab]
0.40–0.50	1.27[a]	1.90[b]	1.47[a]	1.35[a]	1.35[a]

Data followed by the same superscript within a row are not statistically different according to the protected LSD test (P=0.05).

Bulk density and porosity give information on the cultivability, air regime and compactness conditions and on the potentially available moisture content of the soil. The effects of tillage treatments on soil bulk density are shown in Figures 1 and 2. There were no significant tillage effects on bulk density in 1998. The highest bulk density was measured in no-tillage in the second year of the experiment (1998), but when this was compared with ploughing and ridge tillage the difference was not significant (Table 2). The same was true for the ratio of different pore sizes. In the fourth year of the experiment (2000) this tendency changed, and the bulk density in no-tillage increased at a depth of 5–10 cm because of the lack of tillage, while in the case of ploughing and ridge tillage it decreased by 0.1–0.2 $g \cdot cm^{-3}$ compared to the measurements taken two years before (1998). Figures 3 and 4 show the changes in the distribution of pore sizes. The proportion of macropores decreased in no-tillage, so the increase in bulk density and the lack of tillage resulted in a decrease in the macropore ratio. In the sixth year of the experiment (2002) the bulk density was still the greatest in no-tillage, but it was almost the same for conventional tillage; the difference of 0.06 $g \cdot cm^{-3}$ was not significant. The ratio of macropores was still different: a difference of 3.2% was measured between no-tillage and ridge tillage.

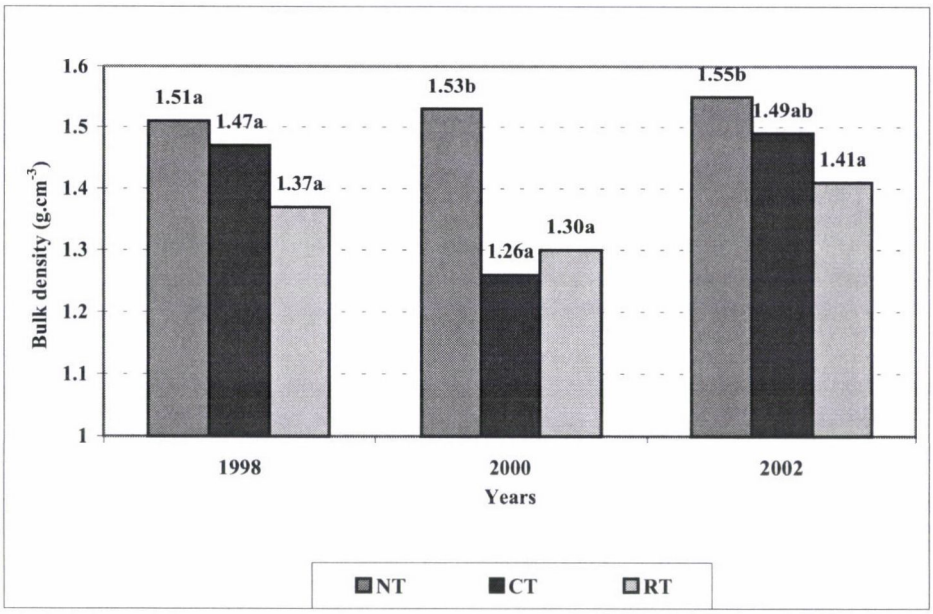

Fig. 1. Soil bulk density in the tillage treatments (no-tillage: NT; mouldboard ploughing in autumn: CT; ridge tillage: RT) at a depth of 0.05–0.10 m

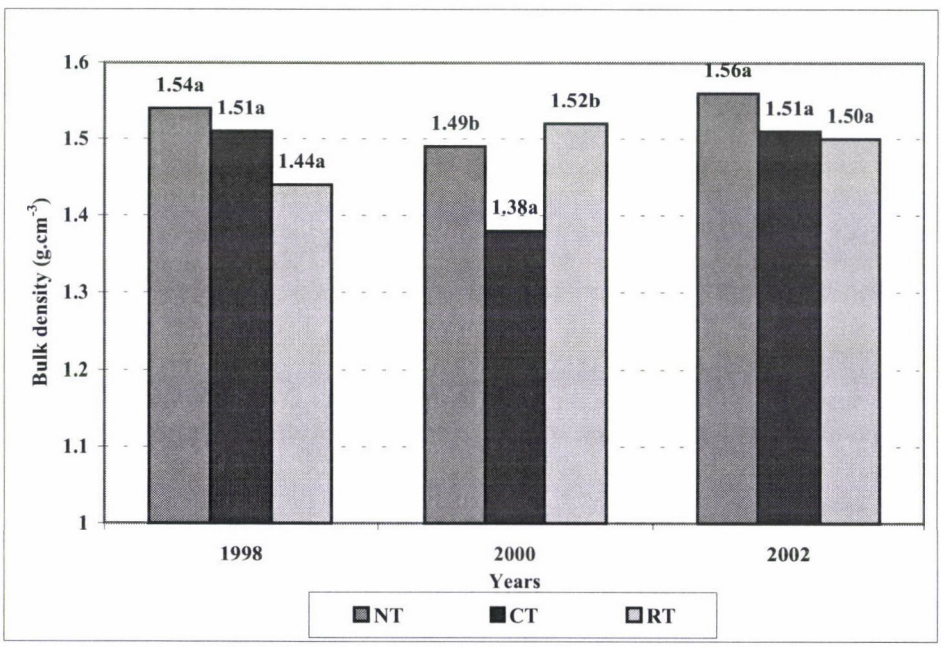

Fig. 2. Soil bulk density in the tillage treatments (no-tillage: NT; ploughing in autumn: CT; ridge tillage: RT) at a depth of 0.15–0.20 m

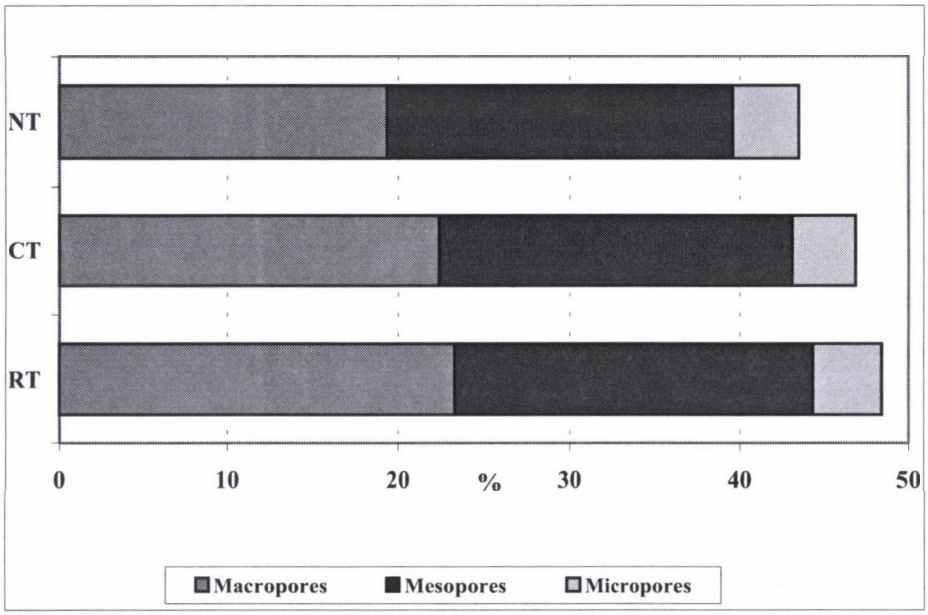

Fig. 3. Soil tillage effects (no-tillage: NT; ploughing in autum: CT; ridge tillage: RT) on pore size distribution as the average of three different measurements at a depth of 0.05–0.10 m

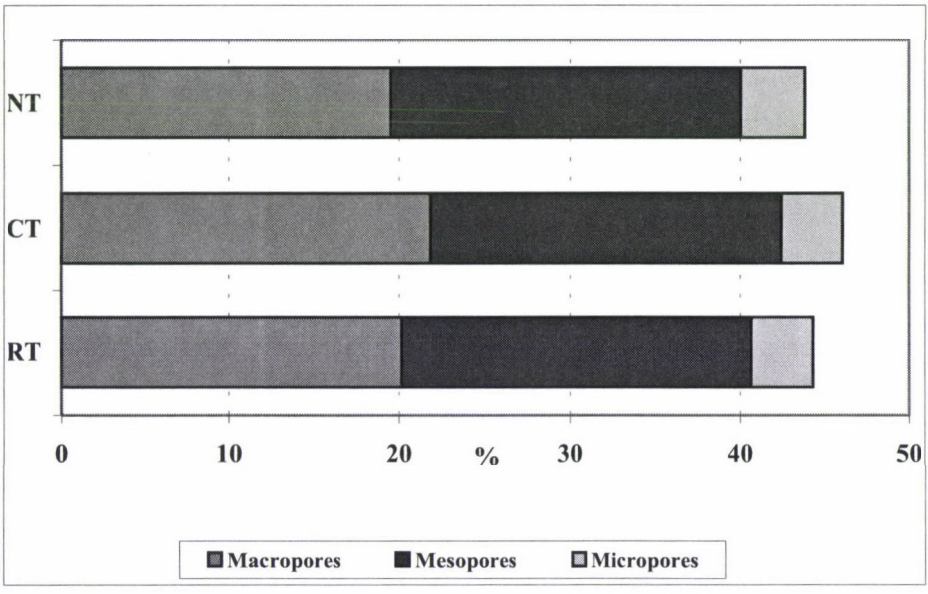

Fig. 4. Soil tillage effects (no-tillage: NT; ploughing in autumn: CT; ridge tillage: RT) on pore size distribution as the average of three different measurements at a depth of 0.15–0.20 m

Table 2
Effect of tillage treatments on soil bulk density ($g \cdot cm^{-3}$) and pore size distribution (%)

Tillage treatments	Bulk density		Macropores		Mesopores		Micropores	
	0.05–0.10	0.15–0.20	0.05–0.10	0.15–0.20	0.05–0.10	0.15–0.20	0.05–0.10	0.15–0.20
1998								
No-tillage	1.51[a]	1.54[a]	20.4[a]	20.1[a]	20.7[a]	20.6[a]	3.9[a]	3.7[a]
Conventional tillage	1.47[a]	1.51[a]	21.3[a]	20.3[a]	20.5[a]	20.0[a]	3.6[a]	3.3[a]
Ridge tillage	1.37[a]	1.44[a]	22.8[a]	20.9[a]	20.5[a]	20.7[a]	4.0[a]	3.6[a]
2000								
No-tillage	1.53[b]	1.49[b]	18.5[a]	20.1[a]	20.1[a]	21.1[a]	4.3[a]	4.1[a]
Conventional tillage	1.26[a]	1.38[a]	25.4[b]	24.0[b]	21.1[a]	21.0[a]	4.1[a]	3.8[a]
Ridge tillage	1.30[a]	1.52[b]	24.9[b]	20.0[a]	22.2[a]	20.4[a]	4.2[a]	3.8[a]
2002								
No-tillage	1.55[b]	1.56[a]	19.0[a]	18.3[a]	20.1[a]	19.9[a]	3.8[a]	4.0[a]
Conventional tillage	1.49[ab]	1.51[a]	20.6[b]	21.2[b]	20.7[a]	21.1[b]	3.6[a]	3.8[a]
Ridge tillage	1.41[a]	1.50[a]	22.2[c]	19.6[ab]	20.8[a]	20.4[a]	3.8[a]	3.9[a]

Data followed by the same superscript within a row are not statistically different according to the protected LSD test (P=0.05).

Discussion

In Austria a large proportion of arable land is located on steep slopes. Many of these soils are highly erodible, and intense spring and summer rainfall is usual. As a consequence erosion can be severe. Ridge tillage, like other conservation tillage systems, maintains a ground cover with less soil disturbance than traditional cultivation, thereby reducing runoff, soil loss and energy use while maintaining crop yields and quality. An experiment in a maize (*Zea mays* L.) monoculture was set up for the first time in 1996. This six-year soil cultivation study, conducted on a Typic Agriudoll sandy loam soil in Austria near Pyhra (Lower Austria), determined the effects of tillage treatments on penetration resistance (PR), soil bulk density (BD) and porosity (P). The treatments were conventional tillage (CT) with ploughing, no-tillage with residues left standing (NT) and ridge tillage (RT). High bulk density and soil strength can lower crop yields by impending root growth and may persist for many years. The ridge tillage system proved to be a useful soil conservation method for sandy loam soil in this region. Adverse soil physical conditions that limit soil water infiltration, root growth and crop yield developed when a no-tillage system was used.

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EFFECTS OF SOIL TILLAGE ON THE YIELD AND QUALITY OF TOBACCO IN CROATIA

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The effect of soil tillage on the yield and quality of flue-cured tobacco was studied from 1998 to 2001 at the Experimental Station of the Tobacco Institute Zagreb in Croatia. The trial soil was a Luvisol with an acid reaction and a low content of colloids and organic matter.

There were three treatments in the experiment:

1. autumn ploughing,
2. spring ploughing, and
3. autumn ploughing + chisel ploughing in spring.

The treatments were arranged in random blocks with four replications. Soil ploughed in both autumn and spring gave higher yields of flue-cured tobacco in comparison with autumn ploughing alone. Soil ploughed only in autumn became compacted due to settling during the winter and spring because of the unstable structure and unfavourable texture.

Keywords: Luvisol, soil tillage, soil resistance, tobacco yield and quality

Introduction

In Croatia, tobacco is grown in the Drava Valley. Due to the small size of many farms and to economic factors tobacco is often grown in a narrow crop rotation or in a monoculture. The high content of silt particles and fine sand in the mechanical composition, combined with shallow tillage, frequently causes an increase in soil compaction in the ploughed and sub-ploughed layers (Turšić et al., 1998). Akehurst (1981) and Hawks and Collins (1983) emphasized that tobacco should be grown on loose soils, with a low content of organic matter and of total and mineral nitrogen, a slightly acid reaction (pH 5.5–6.0) and a fairly light mechanical composition. The use of heavy machinery for soil tillage, particularly during the growing period, increases treads and compacts the soil, which has become a global problem (Soane and Ouwerkerk, 1994). Lower tobacco yields are the result of soil compaction and increased bulk density, which often prevents tobacco roots from penetrating into deeper soil layers (Turšić et al., 1998). Soil compaction is often a limiting factor in tobacco production and it cannot be solved by intensified fertilization (Turšić et al., 2002). The aim of this research was to determine the influence of soil tillage on soil compaction and on tobacco yield and quality under the environmental conditions in Podravina (Northern Croatia).

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Materials and methods

The trial was set up in the autumn of 1998, after the winter wheat harvest, at the experimenal station of the Zagreb Tobacco Institute in Pitomača and included three treatments:

1. autumn ploughing,
2. spring ploughing, and
3. autumn ploughing + chisel ploughing in spring (20 cm).

The soil was ploughed to a depth of 30 cm. Fertilization, cultivation, protection and other agrotechnological measures were performed as usual for tobacco of the Bright Virginia type (flue-cured tobacco) in Croatia.

Field trials were carried out in a random block design with four replications. The size of the experimental plots was 4.4×20 m. The tobacco cultivar in the experimental plots was VaD. The planting interval was 110×45 cm. Four rows of tobacco were planted and the yield and other properties were measured on the two middle rows from each plot. Research was conducted during three vegetation periods (1998/1999, 1999/2000, 2000/2001). Based on the results of soil analysis, the soil was fertilized with 400 kg NPK 7-14-21 fertilizer. The soil resistance and momentary moisture of the soil were measured during tobacco blossoming in the third year of the investigation. The results obtained were statistically analysed using variance analysis.

The experimental soil was a Luvisol (FAO, 1990). Chemical soil analysis was conducted before soil tillage. Particle size distribution (Soil Survey Staff, 1975), physical properties and some chemical properties were determined in each soil horizon. The chemical properties were pH (in KCl and H_2O), organic matter content (ISO, 1996), and the content of available phosphorus and potassium (AL-method; Egner et al., 1960).

The experiments were conducted on a soil typical of the area where tobacco is grown on about 6000 hectares in northern Croatia. According to the texture it was sandy loam in the A_p and E horizons (Table 1). Horizon E had small penetration capacity and was compacted. It had small capacity for water in the A_p horizon and small capacity for air in the E horizon (Table 2). The soil reaction was acid, the content of organic matter low, and the provision of available phosphorus and potassium moderate to good.

The climate conditions during tobacco vegetation had an influence on the yield, especially on the quality of tobacco (Papenfus, 1970; Vepraskas et al., 1987; Vepraskas and Miner, 1987). According to the data obtained from the meteorological station situated in the immediate vicinity of the experimental plot, there were considerable differences in the amount and distribution of precipitation (Table 3). Precipitation of 288.5 mm was recorded during the vegetation period of 1999, 285.5 mm in 2000, and 399.6 mm in 2001. The average amount of precipitation during the tobacco vegetation period (from May to September) over several years was 371.4 mm. The optimal amount of precipitation during the vegetation period for the tobacco growing area in Croatia is about 400 mm.

In all three years there were periods of drought, which were worse in the first two years. No negative temperatures, i.e. no late spring or early autumn frosts, were registered during the tobacco vegetation periods.

Table 1
Mechanical composition of Luvisol from experimental plot, 1998

Soil horizon	Depth (cm)	Percentage of particles				Texture
		Coarse sand (2–0.2 μm)	Fine sand (0.2–0.02 μm)	Silt (0.02–0.002 μm)	Clay (<0.002 μm)	
A_p	0–26	15	58	17	10	sandy loam
E	26–45	18	61	15	6	sandy loam
B_t	45–90	21	36	22	21	loam

Table 2
Physical properties of Luvisol from experimental plot, 1998

Soil horizon	Depth (cm)	Total porosity (vol.%)	Water capacity (vol.%)	Air capacity (vol.%)	Bulk density (g cm^{-3})
A_p	15–20	48.0	33.5	14.5	1.47
E	30–35	39.6	32.0	7.6	1.57
B_t	50–55	42.3	31.0	11.3	1.51

Table 3
Meteorological data, Pitomača, 1999–2001

Month	Year	Air temperature		Sunshine, h	Rainfall, mm	Rainy days, ≥5 mm
		Max.	Min.			
May	1999	18.7	9.0	176	82.9	4
	2000	21.4	10.5	192	19.4	1
	2001	19.7	9.4	207	151.7	9
June	1999	24.1	13.3	234	81.8	5
	2000	24.3	12.5	264	26.7	3
	2001	22.0	12.3	201	59.8	5
July	1999	28.3	15.4	327	33.7	2
	2000	28.9	14.7	338	31.8	3
	2001	27.2	15.0	266	24.9	3
August	1999	24.6	13.0	239	53.6	4
	2000	27.2	13.3	298	114.9	6
	2001	25.2	14.9	215	120.6	8
September	1999	25.8	11.9	223	36.5	2
	2000	21.9	10.2	196	92.7	4
	2001	20.8	10.4	173	42.6	3
Total (V–IX)	1999			1199	288.5	17
	2000			1288	285.5	17
	2001			1062	399.6	28
Average	1970–2001			1155.5	371.4	23

Results and discussion

The time of ploughing had a significant effect on the yield and quality of tobacco in two experimental years (1999 and 2001), but had no impact in 2000. Before the autumn ploughing, directly after the harvest of winter wheat (1998), soil samples were taken for physical analysis and to determine the mechanical soil texture of each horizon. Soil ploughed in the autumn was more compact due to settling during the winter and spring, as indicated by the data on soil resistance shown in Table 4.

The high content of silt particles and fine sand in the soil mechanical composition, combined with shallow tillage, frequently led to an increase in soil compaction in the ploughed and sub-ploughed layers, limiting soil resistance values for root growth range from 2 to 5 MPa.

Table 4
Effect of soil tillage on soil resistance, 25–30 cm, 2001

Treatments	Soil resistance (Mpa)	Soil moisture (%)	Yield average (kg/ha)
Autumn ploughing	5.8*	14.3	2595
Spring ploughing	4.1	12.1	2620
Autumn ploughing+spring chisel ploughing	3.0	13.1	3008*

*$LSD_{5\%}$

Higher soil resistance values were obtained in this trial at bulk densities of 1.4 and 1.6 g cm^{-3}. This led to decrease in the soil micropore content, thereby reducing the aeration and water permeability of the soil. Reduced soil aeration and the infiltration of water reduced root growth and development and nutrient uptake (Soane and Ouwerkerk, 1994).

Tobacco reacted to increased soil compactness with a lower yield (Turšić et al., 1998; 2002). Lower yields of flue-cured tobacco were also obtained in previous, similar experiments involving ploughing depth and subsoiling on compact soils (Turšić, 1989; 1994). Special attention should be paid to tillage and inter-row cultivation, to ensure that the soil is sufficiently loose and aerated in the tobacco rhizosphere (Hawks and Collins, 1983; McKee, 1988; Mihalić et al., 1976; Weybrew et al., 1983). Soil ploughed in autumn, chisel tilled in spring and inter-row cultivated (treatment 3) produced the highest tobacco yield in comparison to soils ploughed only in autumn or spring.

The difference in yield was 21.6% in 1999, 10.3% in 2000 and 15.9% in 2001, or 16% over the three-year average in the case of two ploughings with inter-row cultivation compared to autumn ploughing only.

Statistically significant differences were obtained in two years, while in the third year there was also a tendency to higher yield in the case of two ploughings and inter-row cultivation (Table 5).

This research on Virginia tobacco, as well as previous research on Burley tobacco, has confirmed that increased soil bulk density (compaction) and an unfavourable ratio of macro- and micropores are limiting factors for tobacco yield and quality.

Table 5
Effect of soil tillage on tobacco yield

Treatments	Yield, kg			Mean 1999–2001	Index
	1999	2000	2001		
Autumn ploughing	2686	2748	2351	2595	100
Spring ploughing	2665	2765	2431	2620	101
Autumn ploughing+spring chisel ploughing	3267*	3031	2726*	3008*	116
*$LSD_{5\%}$	432	NS	256	324	

The high content of fine sand and silt particles in the flue-cured tobacco production area in northern Croatia causes frequent crust formation and increased compaction of the ploughed layer. Sub-plough horizons also have increased compaction (bulk density) and reduced permeability due to the unfavourable ratio of macro- and micropores and to long years of shallow soil tillage (Turšić, 1989). Lower tobacco yields are a result of soil compaction and increased bulk density, which often impedes the development of tobacco roots into deeper soil layers. A bulk density of 1.4 g cm^{-3} and a corresponding relative soil compaction of 85% are the threshold value of soil compaction, above which the yields of tobacco and of most other field crops decrease.

The contents of nicotine and reducing sugars were determined on samples from the middle harvest. No significant differences were determined in the content of the above-mentioned chemical substances (Table 6).

Increased soil compaction is very often a limiting factor in the production of tobacco and other agricultural crops, as confirmed by the present research.

Higher fertilizer rates did not result in higher yields in compacted soil (1.6 g cm^{-3}), since the unfavourable physical properties of the soil did not provide the necessary conditions for normal nutrient uptake by tobacco plants.

On the average, there was a correlation between the contents of nicotine and reducing sugars and the amount and distribution of rainfall in the individual phases of tobacco development in the years in which the experiment was conducted (Papenfus, 1970).

Increased nicotine content of tobacco, and a lower content of reducing sugars were recorded in 1999 and 2000, which had less rainfall, fewer rainy days and a worse distribution of rainfall during the maturation and seasoning of picked tobacco leaves.

Conclusions

The soil resistance to penetration was measured during tobacco flowering, and a statistically significant increase in soil resistance (compaction) was recorded in soil tilled only in autumn.

Increased soil compaction considerably reduced the leaf yields and root development of tobacco.

Table 6

Effect of soil tillage on the quality and content of nicotine and reducing sugars in tobacco leaves

Treatments	1999			2000			2001		
	Q	N	R	Q	N	R	Q	N	R
Autumn ploughing	21	2.8	13.7	23	2.7	15.4	29	1.9	19.6
Spring ploughing	20	2.9	13.5	26	2.4	14.9	30	2.0	19.3
Autumn ploughing+spring chisel ploughing	28	3.0	13.6	28	2.8	16.5	35	2.3	19.5
$LSD_{5\%}$	5	NS	NS	NS	NS	NS	4	NS	NS

Q: Quality (% first class value); N: Nicotine %; R: Reducing sugars %; NS: non significant

Soil ploughed in autumn and spring gave higher yields and better quality, with a tendency to higher nicotine content and better reducing sugar content. The soil resistance was lower and the tobacco yield was higher when the soil was ploughed in autumn (30 cm) and tilled with a chisel plough in the spring, as compared to the conventional soil tillage practices in the tobacco production area in Croatia.

Due to increased soil compaction in the subsoil layer (on average below 25 cm), the soils in the tobacco growing region of Croatia (silty texture) require deeper tillage as well as the occasional application of subsoiling as a separate operation.

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EFFECT OF LIGNITE HUMIC ACID ON SOIL NUTRIENT AVAILABILITY AT DIFFERENT GROWTH STAGES OF RICE GROWN ON VERTISOLS AND ALFISOLS

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Field experiments were conducted with rice (ADT-39) during the wet Kharif season (July– October 2001) at two locations, the Tamil Nadu Rice Research Institute (TRRI) farm, Aduthurai (Vertisol) and the Agricultural Research Station (ARS) farm, Pattukkottai (Alfisol), representing the old and new delta areas of the Cauvery, respectively. The same set of treatments was followed in both soils. The treatments consisted of the recommended NPK fertilizer application at 75% and 100% alone, and 10 or 20 kg ha^{-1} humic acid (HA) in combination with NPK fertilizers as soil application, besides an integrated method involving soil application, root dipping and foliar spraying with humic acid and NPK fertilizers. Initial soil samples from the experimental fields were analysed for physical, physico-chemical and chemical properties. Surface soil samples were collected at critical growth stages and analysed for various available nutrients. The results of the field experiments revealed that the application of humic acid along with inorganic fertilizers led to higher soil nutrient availability at all the growth stages of rice. Similar results were obtained in both Vertisol and Alfisol. The present investigation concluded that the best treatment for soil nutrient availability was 10 kg ha^{-1} HA (soil application) + 0.1% HA foliar spray (twice) + 0.3% HA root dipping + 100% NPK, which was on par with the treatment involving 20 kg ha^{-1} HA (soil application) + 100% NPK compared to the other treatments.

Key words: humic acid, rice, potassium humate, nutrient availability

Introduction

Rice is one of the most important food crops in the world, occupying nearly 135 million hectares, making up 28% of total cereals and yielding roughly 370 million tonnes. In India, rice is cultivated on about 38 million hectares, which is about 37% of the total area of cereals (Pandey et al., 2001). The average production of rice in India is very low in comparison to other rice-producing countries. There are many reasons for this low yield, including poor soil fertility. The restoration or maintenance of soil fertility is mainly dependant on the organic matter content of the soil. The integrated use of organic sources and inorganic fertilizers is promising for the achievement of sustainable productivity over a longer period under intensive cropping, besides supplying a satisfactory nutrient turnover in soil-plant systems. Humic substances (humic acid and fulvic acid) are the products of the decomposition processes of organic matter. Humic acid can be applied to soil to improve its fertility, resulting in an increase in crop growth and yields by improving the nutritional status of the soil and plant system.

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Chandrasekaran (1989) reported that the soil application of humic acid as potassium humate might influence crop growth indirectly through the formation of complexes with ammonium, rendering the N slowly available, and also through the formation of complexes with Fe and Zn, rendering them absorbable by plant roots. Raina and Goswami (1988) reported that the humus material increased the P content of the soil due to the prevention of P fixation and also by fixing humophospho complexes, which can be easily assimilated by plants. The humic substances also played an important role in decreasing the phosphate-fixing capacity of highly weathered soils. Humic acid application has both direct and indirect effects on soil nutrient availability, besides resulting in higher crop yields.

In the present investigation, humic acid in the form of potassium humate (a byproduct obtained during coal mining), which is rich in organic carbon content, was applied to rice crops using different methods and at different rates along with inorganic fertilizers. The impact of the treatment on soil nutrient availability was studied in different soils (Vertisol and Alfisol).

Materials and methods

Two field experiments were conducted during the wet Kharif season (July to October 2001) in the Cauvery delta zone of Tamil Nadu, one at Tamil Nadu Rice Research Institute (TRRI), Aduthurai (Soil 1 - Vertisol), representing the soils of the old Cauvery delta, i.e. clay loam soil, and the other at the Agricultural Research Station (ARS), Pattukkottai (Soil 2 - Alfisol), representing the soils of the new Cauvery delta, i.e. sandy loam soil. The experimental design adopted was a randomized block design with seven treatments replicated thrice. In both the experiments the same set of treatments was applied as follows:

T_1 – Control
T_2 – 75% of recommended NPK*
T_3 – 100% of recommended NPK*
T_4 – 10 kg ha^{-1} humic acid (soil application) + 0.1% humic acid foliar spray (twice) + 0.3% humic acid root dipping + 75% of recommended NPK
T_5 – 10 kg ha^{-1} humic acid (soil application) + 0.1% humic acid foliar spray (twice) + 0.3% humic acid root dipping + 100% of recommended NPK
T_6 – 20 kg ha^{-1} humic acid (soil application) + 75% of recommended NPK
T_7 – 20 kg ha^{-1} humic acid (soil application) + 100% of recommended NPK

The characteristics of the experimental fields are given in Table 1.

The humic acid material (potassium humate), a byproduct obtained from lignite, was supplied by the Neyveli Lignite Corporation. The humic acid material was applied to the soil by mixing it with sand and broadcasting it before transplanting the rice seedlings. The whole of the recommended rate of P fertilizer (single superphosphate) and 1/4 of the recommended rates of N (urea) and K (muriate of potash, potassium chloride) were applied as basal fertilizer, while the remaining 3/4 of N and K were applied in three splits, i.e. during the active tillering, flowering and maturity stages. The 30-day-old medium duration rice seedlings (ADT-39) were transplanted to the experimental field with 2–3 seedlings per hill at a spacing of 15×10 cm. A uniform level of 5 cm water was maintained throughout the crop period by regular irrigation. Buffer channels were laid between the plots to avoid the movement of water from one plot to another. The usual management practices (plant protection measures to check pests and diseases, and weed control) were adopted.

* Recommended fertilizer rate for rice (ADT-39) is 100:50:50 kg N, P_2O_5, K_2O/ha.

Table 1
Initial characteristics of the experimental soil

Properties	TRRI-Aduthurai (Vertisol)	ARS-Pattukkottai (Alfisol)
Physical properties		
Clay (%)	34.06	18.57
Silt (%)	16.40	4.78
Fine sand (%)	27.60	27.92
Coarse sand (%)	20.11	47.50
Textural class	Clay loam	Sandy loam
Bulk density (Mg m^{-3})	1.32	1.33
Pore space (%)	46.8	40.19
Physico-chemical properties		
pH	8.4	6.0
EC (dSm^{-1})	0.55	0.14
CEC (cmol(p+) kg^{-1})	23.7	13.6
Chemical properties		
Organic carbon (%)	0.623	0.267
Humic acid content (%)	0.284	0.126
Total N (%)	0.097	0.083
Total P (%)	0.257	0.185
Total K (%)	0.382	0.217
Available N (kg ha^{-1})	234	214
Available P (kg ha^{-1})	21.8	16.5
Available K (kg ha^{-1})	447	264
Exchangeable Ca (cmol(p+) kg^{-1})	13.26	4.08
Exchangeable Mg (cmol(p+) kg^{-1})	7.34	2.97
Exchangeable Na (cmol(p+) kg^{-1})	2.2	0.72
Exchangeable K (cmol(p+) kg^{-1})	1.20	1.60
Micronutrients		
DTPA Zn (ppm)	3.4	3.20
DTPA Fe (ppm)	45.8	32.3
DTPA Cu (ppm)	6.02	5.44
DTPA Mn (ppm)	12.06	7.65

In the root dipping treatment, the rice seedling roots were dipped in 0.3% humic acid solution for half an hour before transplanting. Foliar sprays with humic acid (0.1%) were given twice, first during the active tillering stage, i.e. 20 days after transplanting (DAT), and the second during the flowering stage, i.e. 40 DAT. The foliar sprays were applied in the evening hours.

The soil samples were collected at the tillering, flowering and harvest stages for the analysis of major and micronutrients, and various methods were adopted to analyse the soil samples. The particle size distributions were estimated by the international pipette method (Piper, 1966). Total nitrogen was estimated by Kjeldhal's method (Piper, 1966). Organic carbon content was estimated by the chromic acid wet digestion method (Walkley and Black, 1936). Physico-chemical properties (pH, EC and CEC) and chemical properties (total phosphorus, potassium, calcium and magnesium, exchangeable calcium and magnesium) were estimated according to the procedure outlined by Jackson (1973). Available nitrogen was estimated by the alkaline permanganate method (Subbiah and Asija, 1956). Available phosphorus was estimated by the procedure given by Olsen et al. (1954) and available potassium by the method suggested by Stanford and English (1949). The DTPA-extractable micronutrients (Fe, Mn, Cu and Zn) were estimated using an atomic absorption spectrophotometer. The results of the soil analyses were subjected to statistical scrutiny following the procedure outlined by Gomez and Gomez (1976).

Results

The overall mean of soil available nutrients (N, P, K, Fe, Mn, Zn and Cu) indicated that the clay loam soils of the old delta had higher values, irrespective of stages and treatments, than the sandy loam soils of the new delta. The treatments had a marked effect on the available nutrients at the tillering, flowering and post-harvest stages in both soils. The available nutrient content gradually decreased with the advancement of crop growth due to nutrient removal from the soil by the rice crop.

In the Vertisol, the available N contents at the tillering, flowering and post-harvest stages were 259.8, 242.0 and 230.4 kg ha^{-1}, respectively. From the results of pooled analysis (Table 2), it was found that treatment T7 gave the highest available N content (261.0 kg ha^{-1}), which was on par with T5 (259.6 kg ha^{-1}). In the Alfisol, the available N contents at the tillering, flowering and post-harvest stages was 235.2, 219.0 and 211.5 kg ha^{-1}, respectively, and treatment T7 led to the highest available N (241.4 kg ha^{-1}), which was on par with T5 (240.0 kg ha^{-1}).

In the Vertisol, the available P contents at the tillering, flowering and post-harvest stages were 24.79, 18.57 and 15.36 kg ha^{-1}, respectively. From the results of pooled analysis (Table 3), it was found that treatment T7 gave the highest available P (21.62 kg ha^{-1}), which was on par with T5 (21.22 kg ha^{-1}). In the Alfisol the available P contents at the tillering, flowering and post–harvest stages were 22.46, 18.01 and 16.45 kg ha^{-1}, respectively. From the results of pooled analysis, it was found that treatment T7 resulted in the highest available P (21.16 kg ha^{-1}), which was on par with T5 (21.11 kg ha^{-1}).

Table 2

Effect of treatments on available nitrogen content (kg ha^{-1}) of soils at different growth stages of rice

Treatment	Vertisol				Alfisol			
	Tillering stage	Flowering stage	Post-harvest stage	Mean	Tillering stage	Flowering stage	Post-harvest stage	Mean
T1	221	211	201	211.1	195	181	173	183.2
T2	243	230	221	231.6	221	207	196	208.9
T3	260	243	232	245.0	233	215	206	218.2
T4	266	248	236	249.9	240	229	224	230.9
T5	280	257	242	259.6	257	235	228	240.0
T6	267	249	236	250.6	243	229	222	231.6
T7	282	258	244	261.0	258	237	230	241.4
Mean	259.8	242.0	230.4	244.1	235.2	219.0	211.5	221.9
	CD (P=0.05)				CD (P=0.05)			
T	1.4				1.8			
S	0.9				1.2			
T×S	2.4				3.2			

Table 3
Effect of treatments on available phosphorus content (kg ha^{-1}) of soils at different growth stages of rice

Treatment	Vertisol				Alfisol			
	Tillering stage	Flowering stage	Post-harvest stage	Mean	Tillering stage	Flowering stage	Post-harvest stage	Mean
T1	21.2	14.5	10.3	15.36	15.6	13.2	11.2	13.33
T2	23.9	17.3	14.4	18.53	21.2	17.3	15.8	18.13
T3	24.7	18.7	15.4	19.60	22.4	18.3	16.7	19.12
T4	25.4	19.3	16.2	20.30	24.1	18.7	17.3	20.01
T5	26.1	20.1	17.4	21.22	25.1	19.3	18.3	21.11
T6	25.4	19.3	16.4	20.38	24.0	18.6	17.3	19.96
T7	26.7	20.7	17.5	21.62	24.9	20.0	18.5	21.16
Mean	24.79	18.57	15.36	19.57	22.46	18.01	16.45	18.98
	CD (P=0.05)				CD (P=0.05)			
T	0.20				0.18			
S	0.13				0.12			
T×S	0.35				0.31			

In the clay loam soil, the available K contents at the tillering, flowering and post-harvest stages were 699.9, 663.1 and 634.8 kg ha^{-1}, respectively. From the results of pooled analysis (Table 4), it was found that treatment T7 gave the highest available K (740.66 kg ha^{-1}) followed by T6 (719.11 kg ha^{-1}) and T5 (712.22 kg ha^{-1}). In the sandy loam soil the available K contents at the tillering, flowering and post-harvest stages were 283.52, 251.76 and 222.33 kg ha^{-1}, respectively, and it was found that treatment T7 resulted in the highest available K (279.33 kg ha^{-1}), which was on par with T5 (276.22 kg ha^{-1}).

When the DTPA-extractable micronutrient contents of the post-harvest soil samples were analysed, it was found that the overall values of DTPA-extractable Fe, Mn, Zn and Cu were higher in the clay loam soils of the old delta than in the sandy loam soils of the new delta (Table 5). The treatments had a marked effect on the DTPA-extractable Fe, Mn, Zn and Cu in both soils. In the Vertisol, T7 gave the highest available Fe content of 53.0 ppm, which was on par with T6 (52.6 ppm) and superior to all the other treatments. In the Alfisol treatment T7 again resulted in the highest available Fe content (39.9 ppm), which was on par with T5 (39.8 ppm). A similar trend was observed in the case of available Zn content in both soils. In the clay loam soil, the highest available Mn content was recorded in T7 (14.92 ppm), which was on par with T5 (14.76 ppm), but was superior to all the other treatments. In the Alfisol, T7 led to the highest available Mn content, which was on par with T6 (13.53 ppm), but superior to all the other treatments. In the Vertisol the highest value of available Cu (6.65 ppm) was recorded in treatment T7, which was on par with T6 (6.64 ppm), but superior to all the other treatments. In the Alfisol, the maximum available Cu content was recorded in T7 (6.36 ppm), which was on par with T5 (6.35 ppm), but superior to all the other treatments.

Table 4
Effect of treatments on available potassium content (kg ha^{-1}) of soils at different growth stages of rice

Treatment	Vertisol				Alfisol			
	Tillering stage	Flowering stage	Post-harvest stage	Mean	Tillering stage	Flowering stage	Post-harvest stage	Mean
T1	435	412	401	416.56	206	185	155	181.89
T2	723	681	644	682.89	275	244	216	245.44
T3	733	697	653	694.44	284	257	225	255.33
T4	735	698	655	695.77	294	264	236	264.44
T5	740	704	692	712.22	313	271	243	276.22
T6	742	723	694	719.11	295	264	236	256.11
T7	791	725	706	740.66	316	277	245	279.33
Mean	699.90	663.10	634.86	665.95	283.52	251.76	222.33	252.54
	CD (P=0.05)				CD (P=0.05)			
T	1.88				1.98			
S	1.23				1.30			
T×S	3.25				3.43			

Table 5
Effect of treatments on available micronutrient contents (ppm) of the soil at the post- harvest stage

Treatment	Vertisol				Alfisol			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
T1	42.2	10.56	2.8	5.94	28.5	7.12	2.6	5.37
T2	47.4	12.45	3.5	6.08	33.3	9.40	2.8	5.82
T3	49.2	13.73	4.0	6.20	34.3	10.67	3.1	6.05
T4	51.4	14.23	4.3	6.32	37.8	11.40	3.4	6.15
T5	51.6	14.76	4.5	6.50	39.8	11.53	3.8	6.35
T6	52.6	14.25	4.5	6.64	37.9	13.53	3.4	6.23
T7	53.0	14.92	4.8	6.65	39.9	13.80	3.9	6.36
CD (P=0.05)	0.5	0.18	0.3	0.10	0.3	0.31	0.2	0.15

Discussion

With a view to studying the effect of humic acid (as potassium humate) on nutrient availability in the presence of NPK fertilizers, the available N, P and K were analysed at different crop growth stages, while the content of micronutrients was analysed at harvest.

In the present study, the addition of humic acid was found to increase the availability of major and micronutrients. Due to the application of NPK fertilizers either at 100% or 75% of the recommended dose, the availability of N, P and K in both the experimental soils (Vertisols and Alfisols) at all three growth stages of the rice crop was improved. The soluble forms of nutrients added with the fertilizer may have enriched the NPK content of the soil solution, consequently improving their availability. The addition of humic acid enhances

mineral decomposition, thereby releasing elements from the molecular state to the adsorbed state, which is more readily available to higher plants. The formation of stable organomineral complexes with ions such as Ca^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Mn^{2+} also leads to increased nutrient availability in the soil (Brady, 1996).

The enhanced microbial activity in the presence of humic substances also facilitated the release of N from mineralized soil organic matter, which could explain the increased available N content, as reported by Deepa (2001). The combined application of HA and NPK fertilizers led to a marked increase in the availability of N due to the smaller loss of added N and the regulation of N release at a relatively slow rate (Mallikarjuna Rao et al., 1987).

Similar to N, the availability of P was also increased in both soils. The high available P content of soils treated with HA was ascribed to the tendency of metal humates to extract more P from native sources, leading to the increased availability of P in the soil (Mary et al., 2002). In the presence of humic acid, the P availability was improved due to the chelation of HA with Al, rendering it inactive for reaction with P, thus increasing the P concentration in the soil solution. The results of the present investigation were in agreement with those of Tan and Binger (1986). Lee and Bartlett (1976) also found that the formation of a protective film of HA on adsorbing surfaces in the soil decreased the possibility of P retention and increased its solubility, as confirmed by the increased P availability recorded in the humic acid treatments in the present study.

The availability of K was also improved by humic acid application. This was attributed to the release of K from the minerals as a result of the reaction between soil particles and humic acid. The increase in the available K could be ascribed to the solubilizing effect of the acids produced during the biodegradation of SOM (mainly humic substances) coupled with its release from exchange sites by other cations, such as NH^4, thus enriching the K concentration in the soil solution (Raju and Mukhopadhyay, 1976). The beneficial effect of HA on the available K was due to the reduction of K fixation and the release of K by the interaction of HA with clay minerals, besides its direct contribution to the available K pool of the soil.

The combined application of NPK fertilizer with HA favourably influenced the available N, P and K content of the soil. In the presence of HA the 100% rate of NPK fertilizer increased the available N, P and K status of the soil to a greater extent than the 75% rate. The combination of NPK fertilizer and HA led to better nutrient availability than fertilizer alone. Tandon (1988) observed a similar increase and suggested that the humic substances from decomposed FYM in the soil may have resulted in greater NPK availability. This reasoning is supported by the observations made in the present study. The changes in NPK content indicated a declining trend as the growth of rice advanced towards maturity due to crop nutrient removal and transformation losses. The magnitude of the decline was greater in the NPK treatment than in the NPK + HA treatment, highlighting the more intense depletion pattern in the former than in the latter.

Humus had the ability to hold micronutrients for a considerable time and release them when needed to crops. The increased Fe availability in the HA plots might be due to complex formation between HA and metals, particularly Fe, and its further release on dissolution. This is in line with the findings of Banerjee and Basak (1978). The prevention of the formation of insoluble, immobile hydroxides of Zn also increased the Zn availability in the soils. These results corroborate the findings of Stumm and Morgan (1970).

The increased availability of micronutrients due to the addition of HA, observed in the present study, might be attributed to the ability of humic substances to form chelating compounds, as reported by Elgala et al. (1978) and Shanmugam et al. (1989). The reduced redox potential after humic acid application also increased the Mn, Cu, Fe and Zn availability in the post-harvest soil sample. These findings are supported by the results of earlier investigators (Shuman, 1988).

The application of 10 kg humic acid (soil application) + 0.1% humic acid foliar spray (twice) + 0.3% humic acid root dipping + 100% recommended NPK to the rice crop thus increased nutrient availability in both Vertisols and Alfisols, thereby improving soil fertility and productivity.

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YIELD AND QUALITY OF BABY CORN (*ZEA MAYS* L.) AS INFLUENCED BY SPACING AND FERTILIZATION LEVELS

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A field experiment was conducted on baby corn (*Zea mays* L.) at Bangalore, India during 2001 and 2002 with three spacings and seven fertilization levels to study their effect on the yield and quality of baby corn and green fodder. A wider spacing of 45 × 30 cm significantly increased yield components, sensory and nutritional parameters of baby corn and green fodder compared with closer spacing, while the green fodder yield was significantly higher at a closer spacing of 45 × 20 cm or 30 × 30 cm. The application of 150 : 75 : 40 kg NPK ha^{-1} + 10 t farmyard manure (FYM) was found to be optimal for obtaining high baby corn and fodder yields with good quality.

Key words: baby corn, spacing, fertilization levels, yield, quality

Introduction

Baby corn (*Zea mays* L.) is an offshoot of maize which is grown for its young, fresh, finger-like green ears, harvested at the time of silk emergence and before pollination and fertilization. It is used as a vegetable and in inter-continental dishes, as well as for canning. After the harvest of the baby ears, its lush green, soft, succulent, highly palatable fodder gives an additional income to the growers. Baby corn cultivation is a recent development in India. It is becoming popular among the city elite and the processing industry. Cultivation techniques need to be improved if the potential yield of quality baby corn is to be achieved. Fletcher (1975) opined that baby corn production was similar to that of sweet corn, while Galinat and Lin (1998) obtained higher net returns with a 40 × 20 cm spacing. An increased response to applied nitrogen was observed in baby corn by Pandey et al. (1998) and Sundersingh (2001).

The plants were detasselled and 8–10 harvests of baby corn were made from 68–75 days after planting. The optimum spacing and quantity of fertilizer required to obtain quality baby corn has not yet been studied. Hence, the present investigation was undertaken to determine the optimum requirements for spacing and fertilizers to maximize the crop yield and quality of baby corn.

Materials and methods

The field experiment was conducted during the summer seasons of 2001 and 2002 at the Main Research Station of the University of Agricultural Sciences, Bangalore, India. The soil of the experimental site was well drained sandy loam, low to medium in organic carbon (0.47–0.57%), low in available nitrogen (131.78–161.25 kg ha^{-1}) and available phosphorus (29.63–33.25 kg ha^{-1})

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and medium in available potassium (221.05–249.45 kg ha^{-1}). The pH of the soil ranged from 6.25 to 6.53 during both years of the study. The experiment comprised three spacings 45 × 30 cm (S_1), 45 × 20 cm (S_2) and 30 × 30 cm (S_3) and seven fertilization levels 10 t FYM (F_1), 100 : 50 : 27 kg NPK ha^{-1} (F_2), $F_1 + F_2$ (F_3), 150 : 75 : 40 kg NPK ha^{-1} (F_4), $F_1 + F_4$ (F_5), 200 : 100 : 53 kg NPK ha^{-1} (F_6) and $F_1 + F_6$ (F_7) arranged in a factorial randomized block design (RBD) with three replications in 4.5 × 3.0 m plots. Farmyard manure (FYM) was applied two weeks before sowing and baby corn hybrid PAC–792 was sown on 1 February 2001 and 6 February 2002 as per the treatment schedule. Two plants per hill were maintained. Half the nitrogen (urea) and the full rate of P (single superphosphate) and K (muriate of potash) was given as basal fertilizer, while the remaining N was given at 30 days after sowing (DAS). The crop received weekly irrigations (40 mm) and detasselling was done to avoid pollination. The crop was harvested soon after silk emergence. There were eight pickings during the harvesting period. The yield per hectare was calculated by pooling the yield obtained in the net plot area over the entire harvesting period. After the completion of picking, the fodder was harvested and the yield per hectare was recorded. Observations on yield parameters were made on five tagged plants in the net plot area. Sensory indicators (appearance, colour, texture, taste and juiciness of the baby ears) were evaluated by a panel of judges and the scores were given accordingly. The nitrogen percentage in baby corn was estimated by a modified micro-Kjeldhal method, as outlined by Jackson (1973). The crude protein content was calculated by multiplying the nitrogen percentage by a factor of 6.25 (Humphries, 1956). The reducing sugars in baby corn was estimated by the Somogyic method (Nelson, 1944) and expressed as a percentage. Phosphorus and potassium (Jackson, 1973), calcium and fibre (Piper, 1966) and ascorbic acid were estimated by the 2,6-dichlorophenoindophenol method. The quality of baby corn fodder was assessed with respect to crude protein, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin by the standard procedure. The data were subjected to analysis of variance and the results were interpreted at a probability level of $p < 0.05$.

Results and discussion

Baby corn yield

A significant increase in the baby corn yield was registered at the 45 × 30 cm spacing (77.24 q ha^{-1}) as a consequence of a significant increase in the number of baby ears per plant (2.16), length (22.69 cm), girth (13.06 cm), weight (33.66 g) and yield per plant (75.19 g) as compared to spacings of 45 × 20 cm and 30 × 30 cm (Table 1). In baby corn the wider corridor facilitates detasselling and baby corn harvesting. Two plants per hill at 45 × 30 cm spacing was found to be optimum.

The baby corn yield increased with an increase in the fertilizer level up to 200 : 100 : 53 kg NPK + 10 t FYM ha^{-1}, but the increase was only significant up to 150 : 75 : 40 kg NPK + 10 t FYM ha^{-1} (82.40 q ha^{-1}). This increase in baby corn yield was due to an increase in yield components. On the international market the required standard length of dehusked baby corn is 4–9 cm. In the present study the desired length of dehusked baby corn was achieved with 150 : 75 : 40 kg NPK + 10 t FYM ha^{-1}. The leaf chlorophyll a, b and total at 55 DAS increased with an increase in the fertilizer level, as reflected by the greater accumulation of photosynthates in the baby corn. These results confirm the findings of Sundersingh (2001) who observed comparable yields at 150 and 180 kg N ha^{-1}.

Table 1

Baby corn yield (1; q ha^{-1}), green fodder yield (2; t ha^{-1}), No. of baby ears (3) and the length (cm), girth (cm), weight (g) and yield (g $plant^{-1}$) of husked baby corn as influenced by spacing and fertilization levels (Pooled data of 2001 and 2002)

Treatments	1	2	3	Husked baby corn				Leaf chl.* at 55 DAS		
				Length	Girth	Weight	Yield	a	b	Total
A: Spacing (cm)**										
S_1	77.24	34.75	2.16	22.69	13.06	33.66	75.19	1.03	0.63	1.92
S_2	74.26	38.56	2.04	20.90	12.64	31.21	64.42	1.01	0.61	1.88
S_3	67.56	36.81	1.82	19.58	11.51	28.15	59.00	1.02	0.62	1.88
S.E.	0.75	0.94	0.04	0.38	0.15	0.45	0.94	0.013	0.006	0.014
$LSD_{5\%}$	2.12	2.64	0.10	1.08	0.42	1.27	2.65	NS	0.016	NS
B: Fertilizer levels (kg NPK ha^{-1})										
F_1	43.43	23.93	1.48	14.59	9.63	19.32	32.71	0.87	0.54	1.64
F_2	63.30	35.86	1.88	17.66	10.94	24.63	47.59	0.96	0.58	1.78
F_3	73.43	34.89	2.02	19.44	11.72	27.38	53.56	0.99	0.61	1.87
F_4	78.79	36.50	2.09	22.28	12.69	31.17	67.12	1.02	0.63	1.91
F_5	82.40	40.26	2.14	23.73	13.47	35.02	77.50	1.07	0.65	1.98
F_6	85.17	42.37	2.22	24.57	13.95	39.51	90.73	1.09	0.66	2.02
F_7	85.78	43.16	2.23	25.12	14.41	40.04	94.21	1.11	0.67	2.05
S.E.	1.15	1.43	0.06	0.59	0.23	0.69	1.44	0.019	0.009	0.022
$LSD_{5\%}$	3.24	4.03	0.16	1.65	0.64	1.93	4.04	0.055	0.024	0.062
Interaction (A × B)										
S.E.	2.00	2.48	0.10	1.02	0.39	1.19	2.49	0.034	0.015	0.038
$LSD_{5\%}$	NS	NS	NS	NS	NS	NS	7.00	NS	NS	NS

*chl.: chlorophyll (mg/g) S_1: 45×30; S_2: 45×20; S_3: 30×30; F_1: 10 t FYM; F_2: 100:50:27; F_3: 100:50:27 + 10 t FYM; F_4: 150: 75:40; F_5: 150:75:40 + 10 t FYM; F_6: 200:100:53; F_7: 200:100:53 + 10 t FYM; NS: Non-significant; ** Two plants were maintained per hill

Sensory evaluation

Wider spacing (45 × 30 cm) gave the highest grades in the sensory tests: appearance (3.89), colour (3.58), texture (3.88), taste (3.93) and juiciness (3.92), compared to the other two spacings (Table 2). With regard to the fertilization levels, the best values of appearance (3.92), texture (3.92), taste (4.18) and juiciness (3.65) were obtained when the crop was supplied with 150 : 75 : 40 kg NPK ha^{-1} + 10 t FYM. The colour preferred by the consumer in the international market is creamy yellow. In the present study the colour score was highest (3.76) when only 150 : 75 : 40 kg NPK ha^{-1} was applied.

Nutritional parameters

Wider or closer spacing did not significantly influence the nutritional parameters. Fertilization levels, however, had a significant effect on the quality of baby corn. The application of 10 t FYM alone led to the lowest crude protein (12.70%), phosphorus (0.48%), potassium (2.22%), calcium (0.51%), sugars (0.0093%), ascorbic acid (70.48 mg 100 g^{-1}) and crude fibre (4.53 %) contents. A significant increase in these parameters was recorded up to the application of

Table 2
Sensory parameters of baby corn as influenced by spacing and fertilization levels
(Pooled data of 2001 and 2002)

Treatments	Sensory parameters				
	Appearance	Colour	Texture	Taste	Juiciness
A: Spacing (cm)					
S_1: 45×30	3.89	3.58	3.88	3.93	3.92
S_2: 45×20	3.78	3.48	3.75	3.49	3.63
S_3: 30×30	3.48	3.49	3.63	3.36	3.44
B. Fertilizer levels (kg NPK ha^{-1})					
F_1: 10 t FYM	3.35	3.27	3.20	3.00	2.69
F_2: 100:50:27	3.47	3.38	3.46	3.37	2.89
F_3: 100:50:27 + 10 t FYM	3.55	3.48	3.62	3.47	3.20
F_4: 150:75:40	3.66	3.76	3.79	3.79	3.38
F_5: 150:75:40 + 10 t FYM	3.92	3.72	3.92	4.18	3.65
F_6: 200:100:53	3.60	3.38	3.92	3.96	3.62
F_7: 200:100:53+ 10 t FYM	3.52	3.60	3.75	3.70	3.26

Scale: 5 - excellent; 4 - Very good; 3 - Good; 2 - Fair; 1 - Poor

150 : 75 : 40 kg NPK ha^{-1}, except for reducing sugars and ascorbic acid (Fig. 1). The relatively higher percentage of crude protein and phosphorus was mainly due to the fact that the crop was harvested when its nutritional demands were high, leading to greater nutrient uptake if supplies were adequate. The greater accumulation of total nutrients and the favourable biochemical relations at higher fertilization levels resulted in greater production and translocation of assimilates to the sink (Kamala Kumari and Singaram, 1996). Similarly, the higher calcium content observed at higher fertilization levels was due to the calcium supplied incidentally through single superphosphate.

Green fodder yield and nutritional qualities of the fodder

The green fodder yield varied significantly at different spacing levels. Closer spacing of 45 × 20 cm or 30 × 30 cm led to higher fodder yields of 38.56 and 36.81 t ha^{-1}, respectively, which were on par with each other but significantly superior to the wider spacing of 45 × 30 cm (34.75 t ha^{-1}) (Table 1). The increased fodder yield with narrow spacing was mainly due to the increased plant population per unit land area. The plant population was 111,111 ha^{-1} for 45 × 20 cm and 30 × 30 cm spacings, with two plants per hill, compared to 74,074 plants ha^{-1} for 45 × 30 cm spacing. Increased fodder yield was observed up to the highest fertilization level of 200 : 100 : 53 kg NPK + 10 t FYM ha^{-1} (43.16 t ha^{-1}), but this was on par with 150 : 75 : 40 kg NPK ha^{-1} + 10 t FYM (40.26 t ha^{-1}). This higher fodder yield was due to better growth at higher fertilization levels compared to lower levels.

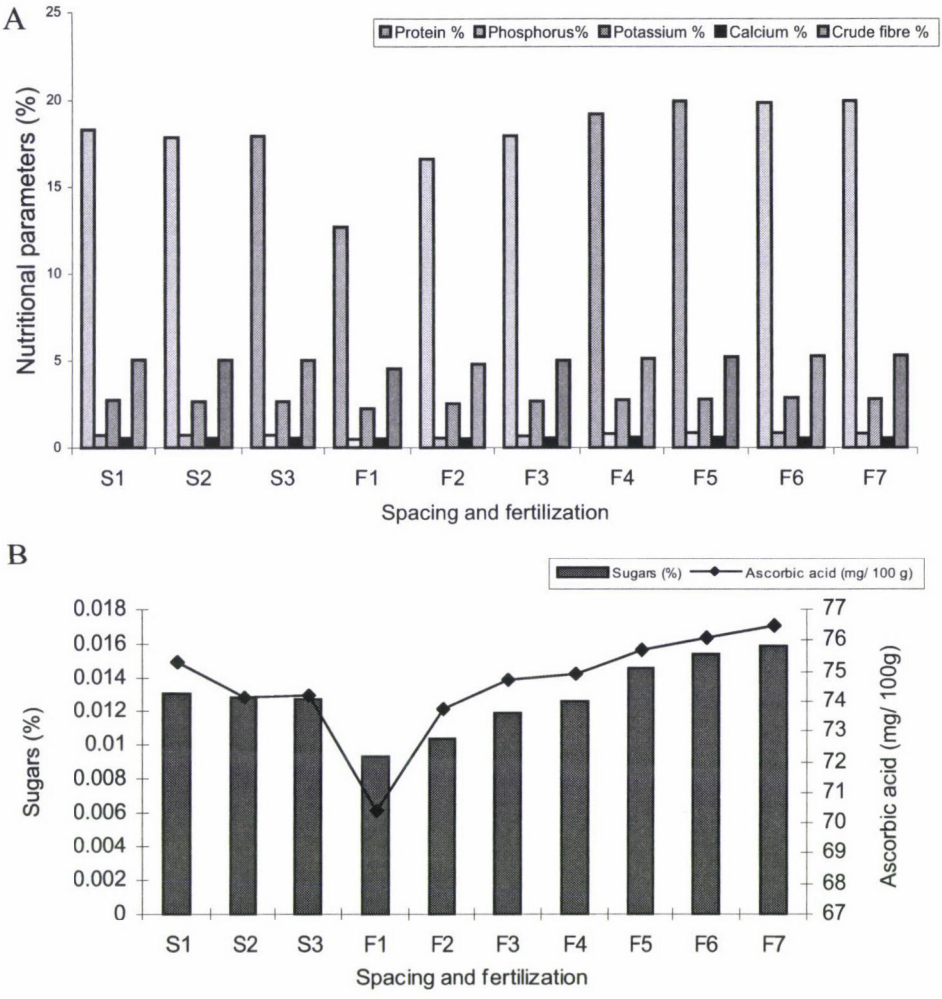

Fig. 1. Nutritional parameters of baby corn as influenced by spacing and fertilization levels
A: Protein, phosphorus, potassium, calcium and crude fibre content; B: Sugars and ascorbic acid (for treatment notes see Table 1)

The wider spacing of 45 × 30 cm caused significantly higher ADF (51.63%) and lignin (9.59%) contents compared to 30 × 30 cm (Table 3). Among the fertilization levels, the application of 200 : 100 : 53 kg NPK + 10 t FYM ha^{-1} resulted in significantly higher crude protein (13.36%), NDF (69.80%), ADF (52.05%), lignin (9.67%) and lignin/ADF ratio (0.19) compared to low fertilization levels, up to 100 : 50 : 27 kg NPK + 10 t FYM ha^{-1}, but was on par with the higher fertilization levels. The digestibility of the fodder depends on the lignin/ADF ratio. The lower the ratio, the better the digestibility, resulting in a higher milk yield. In the present study, there was an increase in the ratio with the increase in fertilization levels and spacing.

Table 3
Nutritional parameters of baby corn green fodder as influenced by spacing and fertilization levels (Pooled data of 2001 and 2002)

Treatments	Crude protein	NDF (%)	ADF (%)	Lignin (%)	Lignin/ADF
A: Spacing (cm)*					
S_1: 45×30	11.49	66.35	51.63	9.59	0.19
S_2: 45×20	11.18	67.43	50.53	8.79	0.17
S_3: 30×30	11.07	67.91	49.36	8.20	0.17
S.E.	0.13	0.58	0.41	0.29	0.01
$LSD_{5\%}$	NS	NS	1.21	0.86	NS
B. Fertilizer levels (kg NPK ha^{-1})					
F_1: 10 t FYM	7.34	66.25	47.41	7.30	0.15
F_2: 100:50:27	9.99	64.52	49.09	8.03	0.16
F_3: 100:50:27 + 10 t FYM	11.10	68.31	50.54	8.80	0.17
F_4: 150:75:40	11.76	65.44	51.04	9.08	0.18
F_5: 150:75:40 + 10 t FYM	12.34	68.88	51.62	9.54	0.18
F_6: 200:100:53	12.82	67.42	51.80	9.59	0.19
F_7: 200:100:53+ 10 t FYM	13.36	69.80	52.05	9.67	0.19
S.E.	0.20	0.62	0.62	0.45	0.01
$LSD_{5\%}$	0.57	1.84	1.84	1.32	NS
Interaction (A×B)					
S.E.	0.35	1.55	1.08	0.77	0.02
$LSD_{5\%}$	NS	NS	3.19	2.29	0.05

NS: Non-significant; *Two plants maintained per hill; NDF: Neutral detergent fibre; ADF: Acid detergent fibre

Considering the yield and quality of both the baby corn and the green fodder, it could thus be concluded that a good yield of quality baby corn combined with green fodder having good digestibility could be achieved by growing baby corn at 45 × 30 cm spacing with 150 : 75 : 40 kg NPK + 10 t FYM ha^{-1}.

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EFFECT OF DATE OF TRANSPLANTING AND NITROGEN ON PRODUCTIVITY AND NITROGEN USE INDICES IN HYBRID AND NON-HYBRID AROMATIC RICE

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A field experiment was carried out during the rainy (*kharif*) season of 2001 at the experimental farm of the Indian Agricultural Research Institute, New Delhi, India, to study the effect of date of transplanting and nitrogen on yield attributes, yields, nutrient accumulation and nitrogen use efficiencies in hybrid and non-hybrid aromatic rice. The experiment consisted of 9 treatments with 2 varieties (Pusa Basmati 1 and Pusa Rice Hybrid 10), 3 transplanting dates (3, 10 and 17 July, 2001) and 4 nitrogen levels (0, 60, 120 and 180 kg N ha^{-1}). Pusa Rice Hybrid 10 had significantly higher values of yield attributes (panicles $hill^{-1}$, panicle weight, spikelets $panicle^{-1}$, filled grains $panicle^{-1}$, 1000-grain weight), yields and nutrient accumulation than the non-hybrid Pusa Basmati 1. There were significant reductions in yield attributes, yields and nutrient accumulation after delayed transplanting. Timely transplanting on 3 July led to 8.4 and 19.1% higher grain yield than transplanting on 10 and 17 July, respectively. Successive nitrogen levels had a significant effect on yield attributes (except 1000-grain weight), yields and nutrient accumulation up to 120 kg N ha^{-1}. The maximum grain yield (5.87 t ha^{-1}) was recorded at the highest level of N nutrition (180 kg N ha^{-1}) and was 4.2, 15.5 and 39.3% higher than in the 120 kg, 60 kg N ha^{-1} and control treatments, respectively. Pusa Rice Hybrid 10 also had significantly higher values of agronomic nitrogen use efficiency (ANUE) (12.5 kg grain kg^{-1} N applied), apparent nitrogen recovery (27.4%), physiological NUE (44.2 kg grain kg^{-1} N uptake), N harvest index (62.7%), N efficiency ratio (119.6 kg dry matter kg^{-1} N uptake) and physiological efficiency index of nitrogen (47.4 kg grain kg^{-1} N uptake) than non-hybrid Pusa Basmati 1.

Key words: hybrid, non-hybrid rice, date of transplanting, nitrogen nutrition, yield attributes, yield, N, P and K uptake, ANUE, ANR, PNUE, NHI, NER and PEN

Introduction

In India the yield of the present high-yielding rice varieties has reached a plateau and plant types with higher yield potential are now needed to overcome this yield stagnation and meet the demands of the ever increasing population. The cultivation of hybrid rice in China has brought great social and economic benefits to the Chinese people (Xizhi and Mao, 1994). Hybrid rice has 20–25% higher productivity potential and could be the key to a second "Green Revolution" in Asia (Lin, 1994). Besides soil and climate factors, management factors also influence fertilizer nitrogen recovery; agronomic nitrogen use efficiency and the physiological efficiency of applied fertilizer have been reported to be about 50% or less for nitrogen, less than 10% for phosphorus and about 40% for potassium (Baligar et al., 2001). Among the different components

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of agronomy packages for rice cultivation, the date of transplanting and nitrogen management are the most important. The performance of rice is greatly influenced by the date of transplanting due to the increase in insect pests and diseases (Halappa et al., 1974). Some scientists reported mid-July as the optimum time for transplanting rice (Gangwar and Sharma, 1997; Nayak and Garnayak, 2000). Rice is planted on the largest area in India and it requires relatively higher doses of N for normal growth and development in comparison with other cereal crops. Most Indian soils are low in N content. The present recommendation for N application to hybrid rice is about 150–200 kg N ha^{-1}, depending on the deficiency in available soil nitrogen. Hence, it is important to calculate the optimum date of transplanting and nitrogen application for newly evolved, scented, high-yielding hybrid and non-hybrid rice cultivars. Keeping this in view an experiment was carried out to study the effect of date of transplanting and nitrogen levels on the productivity, nutrient accumulation (N, P and K) and N use efficiencies of hybrid and non-hybrid aromatic rice under transplanted puddled conditions.

Materials and methods

A field experiment was carried out during the rainy (*kharif)* season (July–November) of 2001 at the experimental farm of the Indian Agricultural Research Institute, New Delhi, India. The soil of the experimental site was sandy clay loam (sand 51.8%, silt 22.1% and clay 26.1%) having pH 8.15, organic carbon 0.54%, available nitrogen 190 kg ha^{-1}, available P 16.8 kg ha^{-1} and available K 316.5 kg ha^{-1} in the ploughed layer. Two rice varieties, Pusa Basmati 1 (traditional high quality aromatic rice variety) and Pusa Rice Hybrid 10 (first aromatic hybrid rice in the world) and 3 dates of transplanting (3, 10 and 17 July, 2001) were assigned to the main plots and 4 nitrogen levels (0, 60, 120 and 180 kg ha^{-1}) to the sub-plots in a split plot design having 3 replications. Both varieties were transplanted at 20 cm × 10 cm spacing keeping 2 seedlings per hill. The total amount of phosphorus, potassium and zinc fertilizers at 60, 40 and 25 kg ha^{-1}, respectively, were applied in the form of single superphosphate, muriate of potash and zinc sulphate before transplanting. Nitrogen as per the treatment was applied as urea in 3 equal splits: 1/3 each at the transplanting, active tillering and panicle initiation stages, respectively. Throughout the growing season the crop was kept in 5–8 cm standing water.

One day before harvesting, 10 hills from each plot were randomly selected for measurements of panicles $hill^{-1}$, panicle length, panicle weight, spikelets $panicle^{-1}$, filled grains $panicle^{-1}$ and 1000-grain weight. On the next day, the net plot area (4.8 m × 1.6 m) of 8 rows from each plot was harvested and their respective weights were recorded for total biomass as well as grain yield estimation (t ha^{-1}). All the plant samples and their parts were oven dried at 60–65°C for 48 hours and ground in a cyclone mill. Before the determination of N, P and K, all the plant samples were moist-ashed with H_2SO_4-H_2O_2. The total nitrogen content in the plant samples was determined by Kjeldahl's method and the total phosphorus content by the colorimetric molybdate yellow colour method. The potassium content was determined by the flame photometry method. The accumulation of N, P and K in the plant samples was estimated from the dry matter accumulation in the respective plant parts in each treatment. The various nitrogen use efficiencies were computed with the formulae given below:

$$\text{Agronomic nitrogen use efficiency (ANUE)} = \frac{Y_t - Y_0}{A_t} \text{ kg grain kg}^{-1} \text{ N applied}$$

$$\text{Physiological nitrogen use efficiency (PNUE)} = \frac{Y_t - Y_0}{U_t - U_0}$$

$$\text{Apparent nitrogen recovery (ANR; \%)} = \frac{U_t - U_0}{N_a} \times 100$$

$$\text{Nitrogen efficiency ratio (NER)} = \frac{\text{Dry matter yield (kg ha}^{-1})}{\text{N accumulated at harvest (kg ha}^{-1}) \text{ (Isfan, 1990)}}$$

$$\text{Physiological efficiency index of nitrogen (PEN)* (Isfan, 1990)} = \frac{\text{Grain yield (kg ha}^{-1})}{\text{N absorbed by biomass (kg ha}^{-1})}$$

$$\text{Nitrogen harvest index (NHI, \%)} = \frac{N_s}{N_t} \times 100$$

where, Y_t = Yield in the test treatment (kg ha^{-1})
Y_0 = Yield in the control (kg ha^{-1})
A_t = Units of nutrient applied in the test treatment (kg ha^{-1})
U_t = Uptake of nitrogen in the test treatment (kg ha^{-1})
U_0 = Uptake of nitrogen in the control plot (kg ha^{-1})
N_a = Nitrogen applied to the test treatment (kg ha^{-1})
N_s = Nitrogen uptake by the grain at harvest
N_t = Nitrogen uptake by the whole plant at harvest

Statistical analysis

All the data recorded in the experiments were subjected to computer analysis using split plot design software.

Results and discussion

Yield attributes and yield

Pusa Rice Hybrid 10 gave significantly higher values of yield attributes (panicles hill^{-1}, panicle length, panicle weight, spikelets panicle^{-1}, filled grains panicle^{-1} and 1000-grain weight) than Pusa Basmati-1, though panicle length remained statistically at par. Lokaprakash et al. (1992) reported significantly higher yield attributes for hybrid rice. There was a significant reduction in yield attributes with a delay in transplanting. Among the six yield attributes, panicles hill^{-1}, panicle weight, filled grains panicle^{-1} and 1000-grain weight showed significant differences between the transplanting dates 3 and 17 July, while a significant linear reduction in the spikelets panicle^{-1} was recorded with each successive delay in the date of transplanting. The reduction in the yield attributes of rice due to delayed transplanting beyond the first week of July was also reported by Singh et al. (1993). Among the yield attributes, panicles hill^{-1} and spikelets panicle^{-1} showed significant differences between the N nutrition levels for successive increases in N levels up to 120 kg ha^{-1}, while panicle length, panicle weight, filled grains panicle^{-1} and 1000-grain weight were significantly different only at higher levels of N nutrition compared with the control (Table 1).

* kg grain kg^{-1} N uptake

Table 1
Effect of variety, date of transplanting and nitrogen on the yield attributes and yields of rice

Treatment	Panicles hill^{-1}	Panicle length (cm)	Panicle weight (g)	Spikelets panicle^{-1}	Filled grains panicle^{-1}	1000-grain weight (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Variety								
Pusa Basmati 1	7.8	26.59	1.59	134.2	92.7	19.10	3.32	6.88
Pusa Rice Hybrid 10	10.2	26.78	2.29	164.3	120.3	21.22	5.87	8.84
CD (P=0.05)	0.61	NS	0.60	4.71	5.67	0.33	0.30	0.57
Date of transplanting								
03.07.2001	9.8	27.26	2.06	157.9	113.4	20.37	4.99	8.00
10.07.2001	8.9	26.53	1.99	151.4	110.1	20.21	4.60	7.98
17.07.2001	8.5	26.27	1.78	138.5	96.1	19.92	4.19	7.59
CD (P=0.05)	0.75	NS	0.22	5.77	6.94	0.41	0.37	NS
Nitrogen (kg ha^{-1})								
0	7.8	25.85	1.76	135.1	97.7	20.50	3.72	6.46
60	8.7	26.49	1.90	145.7	105.5	20.31	4.49	7.73
120	9.6	27.05	2.02	155.9	110.0	20.04	4.98	8.43
180	9.9	27.35	2.08	160.4	112.8	19.80	5.19	8.82
CD (P=0.05)	0.80	0.62	0.16	5.76	5.65	0.36	0.28	0.63

NS = non-significant

Grain and straw yields

There were highly significant differences in grain and straw yields between the hybrid and non-hybrid aromatic rice (Table 1). Pusa Rice Hybrid 10 produced 76% more grain yield (5.81 Mg/ha) than Pusa Basmati 1 (3.32 t/ha), due to the better yield attributes of Pusa Rice Hybrid 10. The present findings are in close agreement with the earlier results of Prasad et al. (1998), who reported a significant increase in both the grain and straw yields of hybrid rice over non-hybrid rice. There was a significant reduction in grain yield with a delay in transplanting, while the straw yield difference between the dates of transplanting remained non-significant. The maximum grain yield (4.99 t/ha) was recorded after transplanting on 3 July, which was 8.4 and 19.1% higher than for transplanting on 10 and 17 July, respectively. The highest grain and straw yields were obtained with the highest level of N nutrition (180 kg ha^{-1}), though the differences between successive N levels were only significant up to 120 kg N ha^{-1}. Similar findings were reported by Hari et al. (1998) and Singh et al. (2000).

Interaction

The interaction effect between the date of transplanting and the variety was found to be significant. Pusa Rice Hybrid 10 produced a significantly lower grain yield with successive delays in transplanting, whereas for Pusa Basmati 1 the differences remained non-significant (Table 2). Similar results were reported by Hari et al. (1997) and Bai et al. (2000). The interaction effect of variety and nitrogen on the grain yield was also significant. Both Pusa Rice Hybrid 10 and

Pusa Basmati 1 produced the maximum yield at 180 kg N ha^{-1}, but the differences were only significant up to 120 kg N ha^{-1} (Table 3). Pusa Rice Hybrid 10 produced a significantly higher grain yield than Pusa Basmati 1 at all levels of N nutrition. These findings are in close agreement with the results of Devaraju et al. (1996).

Nutrient uptake

Nutrient accumulation in plants is a function of nutrient concentration and dry matter accumulation. The hybrid (Pusa Rice Hybrid 10) or non-hybrid (Pusa Basmati 1) nature of the rice had a highly significant effect on the nitrogen, phosphorus and potassium accumulation in the grain, straw and total biomass (kg ha^{-1}). Pusa Rice Hybrid 10 achieved significantly higher nitrogen, phosphorus and potassium accumulation than Pusa Basmati 1 (Table 4), because higher grain and straw yields were recorded for Pusa Rice Hybrid 10.

There was a significant difference in the nitrogen and phosphorus accumulation in the grain yield, but the nitrogen, phosphorus and potassium accumulation in the straw was non-significant. The maximum nitrogen, phosphorus and potassium accumulation was recorded when transplanting was carried out on 3 July. Nitrogen nutrition had a significant effect on the nitrogen, phosphorus and potassium accumulation. With each increment in the nitrogen level there was a significant increase in nitrogen, phosphorus and potassium accumulation up to 120 kg N ha^{-1}, while the maximum nitrogen, phosphorus and potassium accumulation was found at the highest level of nitrogen (180 kg ha^{-1}). Similar results were observed for nitrogen and phosphorus accumulation by Pande et al. (1993).

Table 2
Interaction effect of variety and date of transplanting on the grain yield (t ha^{-1}) of rice

Variety	Date of transplanting		
	03.07.2001	10.07.2001	17.07.2001
Pusa Basmati 1	3.39	3.34	3.22
Pusa Rice Hybrid 10	6.59	5.87	5.16
CD (P=0.05)		0.52	

Table 3
Interaction effect of variety and nitrogen on the grain yield (t ha^{-1}) of rice

Variety	Nitrogen (kg ha^{-1})			
	0	60	120	180
Pusa Basmati 1	2.63	3.24	3.65	3.77
Pusa Rice Hybrid 10	4.82	5.74	6.32	6.61
CD (P=0.05)		0.40		

Table 4
Effect of variety, date of transplanting and nitrogen on N, P and K uptake of rice at harvest

Treatment	N uptake (kg ha^{-1})			P uptake (kg ha^{-1})			K uptake (kg ha^{-1})		
	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total
Variety									
Pusa Basmati 1	42.0	35.3	77.2	8.5	2.7	11.2	13.0	123.5	136.5
Pusa Rice Hybrid 10	78.1	46.0	124.2	15.1	3.4	18.5	23.4	167.9	191.2
CD (P=0.05)	4.1	2.8	6.9	0.7	0.2	0.8	1.3	10.8	12.0
Date of transplanting									
03.07.2001	65.9	41.0	106.9	12.8	3.1	15.8	19.9	148.0	167.9
10.07.2001	60.1	41.6	101.7	11.9	3.1	15.0	18.5	146.9	165.3
17.07.2001	54.2	39.4	93.6	10.8	3.0	13.8	16.1	142.2	158.2
CD (P=0.05)	5.1	NS	8.5	0.8	NS	1.0	1.6	NS	NS
Nitrogen (kg ha^{-1})									
0	47.4	31.7	79.1	9.5	2.5	12.0	14.5	116.9	131.4
60	58.3	39.0	97.3	11.5	3.0	14.5	17.9	141.8	159.6
120	65.8	44.6	110.4	12.8	3.2	16.1	19.8	157.3	177.0
180	68.8	47.4	116.2	13.4	3.5	16.9	20.4	166.9	187.4
CD (P=0.05)	3.8	3.5	6.6	0.7	0.3	0.9	1.3	11.4	12.4

NS = non-significant

Nitrogen use indices

Significantly higher values of ANUE, ANR, PNUE, NHI and PEN were recorded for Pusa Rice Hybrid 10 than for the traditional aromatic rice variety, Pusa Basmati 1. However, Pusa Basmati 1 had significantly higher NER (132.6 kg dry matter kg^{-1} N uptake) due to its low nitrogen uptake and higher dry matter production.

Late transplanting caused a significant reduction in ANUE, ANR, NHI and PNE. Higher values of all these indices were recorded after early transplanting (3 July). The PEN value also decreased with late transplanting but the difference was not significant. Only NER increased with a delay in transplanting, which was due to the higher dry matter production and comparatively lower nitrogen uptake of late transplanted rice.

With each increment of nitrogen, there was a significant reduction in ANUE, ANR and PNUE. Mazid-Miah et al. (1998) reported similar findings. NHI, PEN and NER also decreased at higher rates of nitrogen, the lowest values being recorded at 180 kg N ha^{-1} (Table 5).

Conclusions

Based on the results it can be concluded that the date of transplanting and nitrogen nutrition had a significant influence on yield components, yields and nutrient accumulation (N, P and K kg/ha) in the grain, straw and total biomass. Pusa Rice Hybrid 10 had higher yield potential than the traditional high-yielding aromatic variety Pusa Basmati 1, and also had significantly higher values of yield components, yields and nutrient accumulation (N, P and K kg ha^{-1}). Pusa Rice Hybrid 10 was also found to have superior utilization of nitrogen in terms of ANUE, ANR, PNUE, NHI and PEN compared with the non-hybrid variety, Pusa Basmati 1. In view of these findings, Pusa Rice Hybrid 10 appears to be a promising aromatic rice hybrid.

Table 5
Effect of date of transplanting and nitrogen on ANUE (kg grain kg^{-1} N), ANR (%), PNUE (kg grain kg^{-1} N uptake), NHI (%), NER (kg DM kg^{-1} N uptake) and PEN (kg grain kg^{-1} N uptake) of rice

Treatment	ANUE	ANR	PNUE	NHI	NER	PEN
Variety						
Pusa Basmati 1	8.26	22.87	35.85	54.51	132.60	42.86
Pusa Rice Hybrid 10	12.54	27.44	44.25	62.73	119.63	47.39
CD (P=0.05)	0.76	1.88	3.36	0.55	4.42	1.63
Date of transplanting						
03.07.2001	12.12	29.21	43.40	60.12	124.35	46.11
10.07.2001	9.56	22.77	40.70	58.30	126.12	44.53
17.07.2001	9.52	23.49	36.07	57.44	127.87	44.74
CD (P=0.05)	0.93	2.30	4.11	0.68	NS	NS
Nitrogen (kg ha^{-1})						
0	–	–	–	–	–	–
60	12.70	29.72	41.94	58.97	130.67	46.85
120	10.46	25.13	39.44	58.74	127.36	45.63
180	8.04	20.62	38.79	58.60	124.35	44.34
CD (P=0.05)	0.95	1.57	1.84	NS	4.68	0.51

ANUE = agronomic nitrogen use efficiency; ANR = apparent N recovery (%); PNUE = physiological N use efficiency; NHI = N harvest index (%); NER = N efficiency ratio and PEN = physiological efficiency index of N; NS: Non-significant

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EFFECT OF SEWAGE SLUDGE AND SLAUGHTERHOUSE WASTE COMPOST ON PLANT GROWTH

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Until the 1950s plant nutrition consisted of the application of farmyard manure as part of the within-farm nutritive cycle. As production intensified, artificial fertilizers quickly gained a dominant role. The appearance of environmental damage and the rise in production costs raised the question of the wider application of organic manures. To face this challenge a series of experiments was performed, to examine the possibility of reusing sewage sludge and slaughterhouse waste compost, taking into account the costs and environmental regulations.

At the premises of Beta-Research Ltd. in Sopronhorpács a 2-year plot experiment with 2 factors and 4 repetitions in randomly arranged blocks was carried out in 2000 and 2001. The experimental plant was sugar beet (*Beta vulgaris* L. var. *saccharifera* Alef.) in the first year and spring barley (*Hordeum vulgare* L.) in the second year. The aim of the experiment was to examine the effect of sewage sludge and slaughterhouse waste compost on plant growth and internal parameters and to determine the optimal and unfavourable rates of these manures from the agricultural point of view.

Evaluating the data it was found that the treatments caused statistically significant ($LSD_{5\%}$) differences in yield, sugar content, retrievable sugar content, sugar yield, α-amino-nitrogen and potassium content and recoverability quotient compared to the control.

An analysis of the experimental results demonstrated that 25–50 t/ha sewage sludge application could be recommended under the given field conditions, while compost could be applied at a rate of 150–200 t/ha without any danger of damaging the plants. Several further factors, such as soil, human and animal hygiene and plant nutrition, must also be considered if safe application is to be achieved in the long term.

Key words: sewage sludge, slaughterhouse waste compost, plot experiment, sugar beet, spring barley

Introduction

Civilisation is causing increasingly unfavourable changes in the environment. Parallel to the increase in the population, the economic system constantly inspires an expansion of production and consumption. For this reason the utilization of the environment is growing to an increasing extent (Kádár, 1998; 1999). As a consequence of industrial production and increasing consumer demands the natural, closed ecological system has been disrupted. Nature is unable to make use of many of the materials finding their way into the soil, air and waters. Therefore, the interests of both environment protection and economy urge a minimalization of the use of chemicals and of energy resources originating from industrial processes, and an increase in yields with the

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simultaneous minimalization of risks and costs (Láng and Csete, 1992; Kismányoky and Tóth, 1997). Even if science and technology continue to develop as rapidly as they did in previous decades, the main sources of healthy human nourishment will be agricultural products, the production of which is based on soil fertility (Minyejev, 1988).

Organic fertilization is one of the oldest and most valuable methods of soil cultivation. Its effect is complex and the basic material is a natural product of agriculture (Kismányoky, 1993). Every kind of organic manuring is of overriding importance, because it not only adds nutrients to the soil, but also improves the soil structure and induces useful microbiological processes. Unfortunately, for a long time there has been a shortage of farmyard manure, which is one of the most important kinds of organic manure. Therefore, every possible type of organic manuring is now of vital importance for soil fertility preservation.

When waste water and sewage sludge are applied to arable land, both their water and nutrient contents are utilised (Németh, 1996). They have a favourable effect on the physical, chemical and biological properties of the soil, and the water, nutrients and microbes introduced into the soil cause a considerable change in soil life (Busheé et al., 1998). The fact that they contain nitrogen, phosphorus and potassium in complex form makes them ideal for agricultural use (Allhands et al., 1995). It is a disadvantage that they also contain toxic pollutants, pathogenic microorganisms and highly dangerous salts (Na salts, some hydrocarbonates, sulphates), but if the technologies and legal provisions are strictly adhered to, harmful effects can be prevented (Benedek, 1977; Vermes, 1989).

It is very rarely possible to create an entirely satisfactory soil state suited to plant demands, because of the great number of influencing factors. Therefore, efforts are now focused on creating harmony between plant demands and soil protection requirements in the most efficient way possible (Birkás et al., 2002). As a general principle, after any form of land use it must be possible to restore the complex functions of the soil (Ötvös, 1998). At the same time the decomposing, mobilizing, transforming, etc. roles of the soil must be exploited to meet the demand for an increasing volume of plant production, combined with environment protection. In practice this can be achieved through the controlled recovery of wastes and secondary products and their harmless recycling into the natural cycle (Vermes, 1989).

Materials and methods

A field experiment was set up in plots in Sopronhorpács in 2000–2001 with the cooperation of Beta-Research Ltd. and the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences. The aim was to examine the effect of slaughterhouse waste compost (produced by ATEV Co., Győr) and dewatered sewage sludge from Mosonmagyaróvár on the nutrient supply and soil pollution, and to determine the optimal and

unfavourable doses from the agricultural point of view. Fields with no liming, organic manuring, high rates of mineral fertilization or special cultivation methods were selected for the experiment. Before the experiment was set up, soil samples were taken from the upper, cultivated layer according to the instructions of Ballenegger and di Gléria (1962) and Kádár (1998). The data can be found in Table 1. The field register indicated that winter wheat was produced on this area in 1998–99. The meteorological data characteristic of the experimental period are presented in Table 2.

The plot size, adjusted to the shape of the area, was 40 m^2. The sludge and compost rates applied in the experiment were calculated on the basis of their nitrogen content. The chemical properties of the materials are listed in Table 3, and the rates applied in Table 4.

In the first year the effect of the materials on sugar beet (*Beta vulgaris* L. var. *saccharifera* Alef.) was examined. Sugar beet is known to require a good supply of easily available nutrients. Its nitrogen demand is high, especially at the beginning of development, but it also exhibits a sensitive response to a deficiency of other nutrients (Ruzsányi, 1992; Ragasits, 1994; Pap, 1995). It has proved to be a suitable crop for the disposal of waste water and sewage sludge on agricultural land. Sugar beet seed at a rate of 2.8 U per hectare was sown at a spacing of 45 cm. In the middle of May, when the plants were at the 6–8-leaf stage, the number of plants was thinned by hoeing to achieve 80–90 thousand plants/ha.

The materials to be examined were introduced into the upper 0–20 cm soil layer before sowing, in early spring when the soil thawed out.

Herbicides were applied on three occasions during the vegetation period using *Goltix*, *Dual*, *Betanal Tandem* and *Progress OF*, and hoeing was carried out twice to control weeds. Chemical control against sugar beet flea beetle (*Chaetocnema tibialis* Illig.), using *Mospilan,* was also unavoidable.

Plant surveys, involving canopy development, leaf colour and the degree of canopy closing, were made at germination, at the closing of the canopy and before harvesting. Plant samples, consisting of 20 plants (beet roots and aboveground parts) from each net plot, were taken at harvest. After the necessary measurements (weight of beet roots and aboveground parts), the processing quality of the plants was analysed.

In the second year after-effect examinations were carried out without any further load. In 2001, following the classical crop rotation, spring barley (*Hordeum vulgare* L. cv. Jubilant) was sown. Sugar beet is traditionally the best forecrop for spring barley, because it leaves the soil in good condition, without weeds, and the effect of the deep cultivation favours the faster, deeper rooting of spring barley (Kismányoky, 1992).

Sowing was carried out with 230 kg certified, treated seeds per hectare. Chemical weed control was carried out using *Lintur*, *Juwel*, *Mospilan* and *Sherpa*. Plant surveys were done at the end of tillering (degree of tillering), at heading and at harvesting (stage and colour of plant). Samples of plants harvested at full maturity were taken from 4-metre rows (0.5 m^2) from each net plot. The aboveground plant parts were cut off close to the ground and dried to the air-dry stage. The dry weight, number of spikes and spike and seed weight were recorded. The seed and straw parts were examined after the milling of the samples.

The data gained during the experiment were processed with the Microsoft® Excel 2002 program. The data were evaluated on the basis of Sváb (1981) using bifactorial ANOVA for a randomised block design.

Table 1

Data of preliminary soil analysis on the plot experiment in Sopronhorpács in 2000–2001

pH		K_A	$CaCO_3$	Humus	Tot. N	AL*			KCl*	EDTA*			
H_2O	KCl		%	%	%	P_2O_5	K_2O	Na	Mg	Zn	Cu	Mn	Fe
6.6	5.7	45	0.0	2.0	0.1	158	76.5	19	350	2.0	4.2	280	376

*-extractable; unit of measurement: mg/kg

Table 2
Meteorological data recorded in the Sopronhorpács region in 2000–2001
(source: Beta Research Ltd.)

Month	Rainfall mm	Average temp. °C	Rainy days	Sunny hours	Absolute, °C	
					Min.	Max.
January	24.7	–0.2	10	55.3	–13.9	11.0
February	7.4	3.8	8	136.7	–4.8	14.9
March	67.4	6.2	16	141.0	–4.2	22.0
April	32.4	13.4	7	231.5	–1.8	27.5
May	25.6	17.1	8	263.7	3.2	30.5
June	16.1	20.7	7	300.3	6.2	37.4
July	110.9	18.6	18	188.1	7.6	34.6
August	67.1	21.6	7	272.5	7.0	37.1
September	51.4	15.2	9	152.4	6.2	28.5
October	92.4	12.6	11	122.8	0.1	26.5
November	50.9	8.1	17	65.0	–1.1	20.0
December	37.7	1.8	17	22.5	–10.6	11.5
Total/average for 2000	*584.0*	*11.6*	*135*	*1951.8*	*–13.9*	*34.6*
January	13.9	0.3	15	29.5	–10.9	12.0
February	12.2	3.5	6	98.2	–8.6	17.5
March	45.9	7.4	17	96.9	–4.4	21.5
April	32.9	9.6	12	168.6	–3.6	26.4
May	34.5	17.1	8	274.0	6.0	30.5
June	47.8	18.1	10	219.9	4.8	31.8
July	80.0	21.1	15	230.2	8.8	35.0
August	23.7	21.7	5	274.2	7.2	35.0
September	90.0	13.6	17	121.0	2.6	25.6
October	18.0	13.2	10	122.3	2.5	26.5
November	21.3	4.0	10	68.1	–5.4	14.5
December	29.6	–2.7	11	35.8	–18.5	7.0
Total/average for 2001	*449.8*	*10.6*	*136*	*1738.7*	*–18.5*	*35.0*

Table 3
Chemical composition of the materials added to the soil in the first year of the plot experiment in Sopronhorpács, 2000–2001

	Unit of measurement	Compost	Sewage sludge
Dry weight	%	67.3	20.7
pH		8.4	6.7
Ca	mg/kg dry weight	141600.0	43000.0
Cd	mg/kg dry weight	0.04	0.8
Cr	mg/kg dry weight	10.2	64.9
Cu	mg/kg dry weight	10.6	39.2
Fe	mg/kg dry weight	4905.0	17080.0
Mg	mg/kg dry weight	17650.0	7000.0
Mn	mg/kg dry weight	77.6	186.8
Ni	mg/kg dry weight	4.4	20.3
Pb	mg/kg dry weight	3.5	26.1
Zn	mg/kg dry weight	70.9	285.8
Total N	mg/kg dry weight	11320.0	37000.0
P	mg/kg dry weight	5530.0	14840.0
K	mg/kg dry weight	1565.0	2570.0

Table 4
Quantity of sewage sludge and slaughterhouse waste compost applied in the plot experiment in Sopronhorpács, 2000–2001

Treatment	Material, t/ha	Load dry weight, t/ha	N load, kg/ha
Compost rates (for 2 years)			
1.	Control	–	–
2.	25	16.4	201
3.	50	32.8	402
4.	100	65.6	804
5.	200	131.2	1608
Sludge rates (for 2 years)			
1.	Control	–	–
2.	25	5	185
3.	50	10	370
4.	100	20	740
5.	200	40	1480

Results and discussion

The experimental years were characterized by rainfall deficiency (see Table 2). In the first year withering and a deficiency in the number of plants were observed even on the control plots. In spite of the unfavourable weather, the sugar beet grew well enough on the treated plots, but the larger nitrogen doses caused an oversupply, so that although the root yield was high, the quality parameters deteriorated.

The sugar beet samples taken from the net plots were examined using the Dutch VENEMA method. Measurements were made on the yield per hectare, the sugar content, the retrievable sugar content and sugar yield, the components detrimental to retrievability (α-amino-nitrogen, sodium and potassium content) and the recoverability quotient (Q %).

As shown in Table 5, the increase in sugar beet yield caused by the treatments was statistically significant. The 100 t/ha and 200 t/ha rates of compost and the highest (200 t/ha) rate of sewage sludge caused the greatest changes in the yield. The compost treatments had no significant effect on the sugar content of the beet. However, increasing rates of sewage sludge had a negative effect (in contrast with its effect on the yield), because there was a significant reduction in the digestion value (sugar content) of the sugar beet. Similar results were obtained for the retrievable sugar content (which can be calculated from the sugar content and the components detrimental to retrievability). Even higher rates of compost had a decreasing tendency, but sewage sludge led to a severe reduction in the retrievable sugar content. In the case of retrievable sugar yield (obtained by multiplying the retrievable sugar content by the yield) sewage sludge at a rate of 25 t/ha increased it to the highest degree, but not significantly. There was a downward tendency at higher rates of application. Among the components detrimental to retrievability, there was no

difference in the sodium content, but significant differences were found for the α-amino-nitrogen and potassium contents. Higher rates caused a considerable increase in the α-amino-nitrogen content, which can be explained by the high nitrogen content of sewage sludge and slaughterhouse waste compost. The increase in the potassium content as a result of high rates of sewage sludge application was also statistically significant. Despite the fact that the retrievable sugar yield consistently rose, the cumulated indicator of quality-damaging parameters (dense liquid purity quotient, Q %) cannot be disregarded. The value of this quotient declined as the result of both types of manure.

In the second experimental year, in 2001, the spring barley cultivar Jubilant was examined. As reported in the literature, the water demand of spring barley is less than that of other cereals and much depends on the number of rainy days, besides the actual amount of rainfall. Any rain falling from the waxy ripe stage until full maturity has a negative effect (Lőrincz, 1984; Kismányoky, 1992). The optimal rainfall distribution determined by Lőrincz (1984) is: 30–40 mm in March, 40–50 mm in April, 60–65 mm in May, 50–60 mm in June and 20–25 mm in the first half of July, giving a total of 200–240 mm during the vegetation period. By contrast (as seen in Table 2) there was only 173.1 mm rainfall during the 2001 vegetation period in Sopronhorpács and its surroundings. To make matters worse, 70% of this amount (114.3 mm) fell within 10 days.

Table 5

Influence of treatments on plant parameters and the significance of ANOVA F values in a sugar beet plot experiment in Sopronhorpács in 2000

Treatments		Yield t/ha	S. c. %	Retrievable		K	Na	α-amino-N	Q %
				S. c. %	S. y. t/ha	mmol/kg	mmol/kg	mmol/kg	
Sewage sludge	0	41.08	15.71	13.31	5.49	4.85	0.66	0.50	93.25
	25	53.58	15.56	13.25	7.20	5.12	0.59	0.74	93.20
	50	53.38	15.35	12.80	6.85	5.39	0.90	1.13	92.22
	100	60.38	13.62	10.50	6.49	6.72	0.99	1.89	88.73
	200	56.38	12.48	9.29	5.24	6.79	1.00	2.46	87.10
Slaughterhouse	0	50.80	15.08	12.84	6.55	4.96	0.60	0.38	93.55
waste compost	25	52.10	15.71	13.34	6.98	5.31	0.63	0.53	93.28
	50	58.90	15.63	13.18	7.76	5.21	0.90	0.70	92.94
	100	63.53	15.09	12.43	7.90	5.78	0.83	1.07	91.86
	200	73.50	14.53	11.82	8.71	5.52	0.86	1.66	90.96
$LSD_{5\%}$		12.24	1.34	1.59	2.10	1.12	0.41	0.41	1.77
Source of variation	DF	F value							
Treatment	9	4.16^{**}	5.32^{***}	6.24^{***}	2.15^{*}	3.12^{*}	1.35^{NS}	23.37^{***}	12.75^{***}
Org. fertilizer	1	6.51^{*}	5.19	6.64^{*}	8.37^{**}	2.96^{+}	0.51^{NS}	27.85	17.48
Rate	4	6.34^{***}	7.94	9.68^{***}	1.02^{NS}	4.84^{**}	2.72^{+}	43.07	20.23
Fert. × rate	4	1.39^{NS}	2.74^{*}	2.70^{+}	1.72^{NS}	1.43^{NS}	0.19^{NS}	2.55^{*}	4.08^{*}

S. c.: Sugar content; S. y.: Sugar yield; NS: not significant; + significant at P = 0.1; * significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.001

Due to the lack of rainfall, drought caused scorched spots, and the plant growth and development was retarded. This led to a wide dispersion of the measured values, so statistically significant differences in dry weight, spike and seed weight, and spike number could not be demonstrated (see Table 6).

Because of the extremely dry weather it was difficult to achieve the original experimental aim of establishing an optimal application rate. Nevertheless, it was estimated from the results that the optimal rate for municipal sewage sludge was between 25 and 50 t/ha under the given field conditions. The results obtained in previous years, when there was more rainfall, suggested that 50 t/ha could be applied, but taking into consideration the drier period after 2000, the application of only 25 t/ha is recommended to avoid harmful effects. Before land is treated with sewage sludge, additional analytical and microbiological examinations must be carried out, and soil scientists should be consulted.

Slaughterhouse waste compost caused no yield deficiency even at the 200 t/ha rate and the maximum sugar yield was recorded at this level. The yield was 1.5 times higher than that of the control plots and the differences were significant at the $LSD_{5\%}$ level, but the quality indexes steadily deteriorated.

Table 6

Influence of the treatments on plant parameters and the significance of ANOVA F values in a spring barley plot experiment in Sopronhorpács in 2001

Treatments		Dry weight g/plant	No. of spikes/ plant	Spike weight g/plant	Seed weight t/ha
Sewage sludge	0	559.25	342.75	317.50	5.09
	25	498.50	311.00	284.50	4.71
	50	494.50	330.00	286.75	4.65
	100	511.00	306.75	294.50	4.80
	200	556.25	367.75	331.00	5.46
Slaughterhouse	0	578.25	364.50	326.50	4.92
waste compost	25	533.50	333.75	315.50	5.21
	50	477.75	314.75	282.50	4.43
	100	485.50	297.25	280.00	4.42
	200	579.25	355.50	337.25	5.50
$LSD_{5\%}$		*121.43*	*56.46*	*62.64*	*1.03*
Source of variation	DF		F value		
Treatment	9	0.86^{NS}	1.64^{NS}	1.06^{NS}	1.20^{NS}
Org. fertilizer	1	0.07^{NS}	0.01^{NS}	0.16^{NS}	0.05^{NS}
Rate	4	1.72^{NS}	3.20^{+}	2.02^{NS}	2.22^{+}
Fert. × rate	4	0.20^{NS}	0.48^{NS}	0.31^{NS}	0.46^{NS}

NS: not significant; $^{+}$ significant at P = 0.1; * significant at P = 0.05; ** significant at P = 0.01; *** significant at P = 0.001

Since both the yield and the retrievable sugar yield increased significantly at the highest (200 t/ha) rate of compost application compared to the control, this rate can be recommended. Under the given conditions, the optimal rate cannot be established on the basis of the plot experiment alone. Before the application of this large quantity is commenced, additional analytical and microbiological examinations must be carried out, and soil scientists must be consulted. The critical load cannot be determined from the present results.

The plant surveys revealed that both sewage sludge and slaughterhouse waste compost had similar favourable effects on the soil.

Because of the difficulties involved in the transportation of large volumes of organic manures, they can only be applied economically close to where they are produced. Nevertheless their application is definitely recommended because they are important sources of various nutrients essential for the growth and development of the plants. Because of their fixed form, mineralization takes place gradually, so the nutrients are available to the plants over a longer period and there is less risk of nutrient leaching. In the course of mineralization, more favourable forms are produced. Furthermore, thanks to their complex form they contain many of the macro- and microelements essential for plant development, which are not contained in mineral fertilizers.

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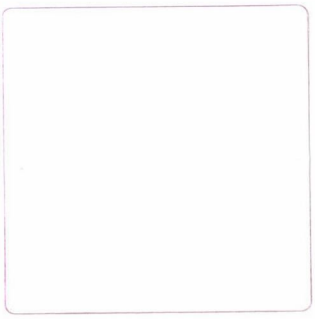

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ASSESSING HERITABILITY AND VARIANCE COMPONENTS OF AGRONOMIC TRAITS OF FOUR ALFALFA (*Medicago sativa* L.) CULTIVARS

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This research was conducted between the years 1999–2002 in the experimental area of the Field Crops Department of Tekirdağ Agricultural Faculty in Turkey. The experiment was laid out in a randomized block design with three replications. Four alfalfa cultivars were used. Variance components, variance coefficients and heritability values were determined for morphological characters, herbage yield, dry matter yield and seed yield. The maximum main stem height (78.69 cm), main stem diameter (4.85 mm), leaflet width (0.93 cm), seeds/pod (6.57), herbage yield (75.64 t ha^{-1}), dry matter yield (20.06 t ha^{-1}) and seed yield (0.49 t ha^{-1}) were obtained from the cultivar Marina. The leaflet length ranged from 1.65 to 2.08 cm and the raceme length from 3.15 to 4.38 cm in the alfalfa cultivars. The highest 1000-seed weights (2.42–2.49 g) were found in cultivars Marina and Sitel. The heritability values of main stem height, main stem diameter, leaflet length and width, leaf/stem ratio, racemes/main stem, raceme length, seeds/pod, 1000-seed weight, herbage yield, dry matter yield and seed yield were calculated as 91.0%, 97.6%, 81.8%, 88.8%, 90.4%, 28.3%, 99.0%, 99.2%, 88.0%, 97.2%, 99.6% and 95.4%, respectively.

Key words: heritability, variance components, alfalfa, *Medicago sativa* L., agronomic traits

Introduction

The methods employed by breeders to improve the productivity and value of alfalfa (*Medicago sativa* L.), are based upon a knowledge of the crop's mode of reproduction and genetic structure. Alfalfa is a naturally out-crossing perennial that depends upon bees for pollination. The flower is complete, therefore selfing can occur. In nature, however, the frequency of selfing is usually much less than that of crossing. Limited cross- and self-pollinations can be made easily by the plant breeder. Some plants are self-sterile or self-incompatible, a few are pollen-sterile, and a very few are ovule-sterile. Alfalfa can be propagated by rooted stem cuttings (Busbice et al., 1972).

Alfalfa is a polymorphic species, adapted to many soils and climates. Inherent variation is immense; the introgression of *M. falcata* into *M. sativa* has increased the genetic variation and range of adaptation. Alfalfa is grown extensively in the temperate climates of all continents (Busbice et al., 1972; Rumbaugh et al., 1988).

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Alfalfa breeders must concentrate on herb and seed yield, hardiness, nitrogen fixation, quality, growth after cutting, longevity, feeding value and other agronomic traits. These components exhibit a continuous range of expression and are quantitatively inherited. The expression of this quantitative inheritance is probably also influenced by the environment. Breeders aim to quantify the impact of genetics and the environment. To help breeders distinguish between genotype and environmental effects, a heritability value (h^2) can be determined using the ratio of genotypic and phenotypic variation (Stoskopf, 1993; Tekeli and Ateş, 2002a; b).

The aim of this study was to determine the heritability and variance components of agronomic properties in four alfalfa cultivars.

Materials and methods

The investigation was carried out in 1999–2002 on clay soil with pH 7.1 on the experimental area of Tekirdağ Agricultural Faculty, in Trakya University located at 41.0°N, 27.5°E, about 5 m altitude above sea level, with a typical subtropical climate. The soil of the experimental area was clay, low in organic matter (1.17%), moderate in phosphorus content (67.4 kg ha^{-1}), but rich in potassium content (644.4 kg ha^{-1}). The total rainfall was 482 mm, 511 mm and 495 mm in the experimental years, compared with the long-term (1989–98) mean of 444 mm. The monthly average temperature (first year 16.4°C; second year 15.7°C; third year 16.9°C) and relative humidity (first year 85%; second year 88%; third year 87%) means were similar to the long-term average (15.5°C; 84%).

Four alfalfa cultivars were used in the study. Cultivar Elçi was obtained from the Agricultural Faculty of Ankara University (Turkey), while the other three (Bella, Marina and Sitel) were obtained from Barenburg Research, Wolfheze, Netherlands. Plots were 5.0×2.0 m, arranged in a randomized block design with three replicates (Gomez and Gomez, 1984). Each plot consisted of 10 rows 20 cm apart and 5 m in length. The seeds were sown at a rate of 1 g m^{-2} (Ateş and Tekeli, 2001) on October 28th 1999 with a hand-seeder. Measurements were made in 2000, 2001 and 2002. The plots were not irrigated or fertilized after sowing and cutting. Three cuts were taken each year at the full-bloom stage. The cutting height was approximately 8–10 cm above ground level.

The main stem height (cm), diameter of main stem (mm), leaflet width and length (cm), leaf/stem ratio, raceme length (cm) and number of racemes per main stem were determined on ten randomly chosen plants. Seeds/pod and 1000-seed weight (g) were measured on ten randomly selected plants when the pods matured (Barnes and Sheaffer, 1995). The main stem diameter was determined between the fourth and fifth nodes. Leaflet width and length were measured on the middle leaflet of the leaf at the fifth node on ten plants (Tekeli and Ates, 2003a). Approximately 500 g samples were dried at 78°C for 24 h to determine the dry matter content and calculate the dry yield (t ha^{-1}) (Tekeli and Ates, 2003b). When the seeds matured, 2 m^{-2} were harvested to determine the seed yield (t ha^{-1}).

The results were analysed using the TARIST statistical program (Açıkgöz et al., 1994). Variance components, genotypic variance coefficient (GVC), phenotypic variance coefficient (PVC) and heritability values (h^2) were calculated according to the equations reported by Kempthorne (1957), Comstock and Moll (1963) and Orak (2000).

Results and discussion

The results of analyses for the traits investigated are given in Table 1. The heritability values (h^2), phenotypic variance (Vp), genotype × year variance (Vgy), genotypic variance (Vg), phenotypic variance coefficient (PVC) and genotypic variance coefficient (GVC) for the cultivars are given in Table 2.

Table 1
Morphological characteristics and herbage, dry matter and seed yield of alfalfa cultivars

Cultivars	2000	2001	2002	Average	2000	2001	2002	Average
	Main stem height (cm)				Main stem diameter (mm)			
Marina	78.03	83.23	74.83	78.69a	4.85	4.88	4.83	4.85a
Sitel	67.75	66.93	68.03	67.57b	4.49	4.38	4.37	4.41b
Bella	57.40	53.30	53.13	54.61d	3.62	3.40	3.11	3.38c
Elçi	61.90	59.03	61.77	60.90c	3.91	4.09	4.17	4.06b
LSD	Cultivars: 6.26 ** Years: ns				Cultivars: 0.38** Years: ns			
	Leaflet width (cm)				Leaflet length (cm)			
Marina	0.89	0.94	0.96	0.93a	2.19	2.01	2.03	2.08a
Sitel	0.80	0.80	0.68	0.76c	2.13	1.95	2.03	2.04a
Bella	0.71	0.68	0.64	0.67d	1.66	1.63	1.66	1.65b
Elçi	0.88	0.84	0.85	0.86b	1.90	1.91	1.90	1.90a
LSD	Cultivars: 0.05** Years: ns				Cultivars: 0.24** Years: ns			
	Leaf/stem ratio				Number of racemes per main stem			
Marina	1.22	1.20	1.24	1.22a	16.63	16.50	14.34	
Sitel	0.95	1.02	1.02	0.99b	13.73	13.80	13.37	
Bella	1.20	1.20	1.18	1.19a	12.73	12.30	11.99	
Elçi	1.03	0.91	0.87	0.94b	15.13	14.30	14.22	
LSD	Cultivars: 0.14** Years: ns				Cultivars: ns Years: ns			
	Raceme length (cm)			Average	Number of seeds per pod			Average
Marina	4.33	4.41	4.42	4.38a	6.57	6.62	6.53	6.57a
Sitel	4.22	4.17	4.22	4.20a	5.97	6.10	6.12	6.06b
Bella	3.12	3.13	3.19	3.15b	3.96	3.98	4.26	4.07d
Elçi	4.44	4.24	4.12	4.27a	4.80	4.74	4.92	4.82c
LSD	Cultivars: 0.23** Years: ns				Cultivars: 0.32** Years: ns			
	1000-seed weight (g)				Herbage yield (t ha^{-1})			
Marina	2.48	2.46	2.53	2.49a	70.10	76.58	80.23	75.64a
Sitel	2.46	2.39	2.42	2.42a	57.70	58.93	61.05	59.23b
Bella	1.90	1.96	1.96	1.94b	43.25	42.20	41.10	42.18d
Elçi	2.02	2.11	2.23	2.12b	46.75	46.58	48.27	47.20c
LSD	Cultivars: 0.25** Years: ns				Cultivars: 2.83** Years: ns			
	Dry matter yield (t ha^{-1})				Seed yield (t ha^{-1})			
Marina	19.51	20.41	20.25	20.06a	0.48	0.49	0.49	0.49a
Sitel	16.71	16.91	16.80	16.81b	0.41	0.40	0.42	0.41b
Bella	13.27	13.05	13.16	13.16d	0.30	0.30	0.30	0.30d
Elçi	14.67	14.58	14.46	14.57c	0.34	0.35	0.34	0.34c
LSD	Cultivars: 0.65** Years: ns				Cultivars: 0.03** Years: ns			

**: P<0.01, ns: non-significant; Average values designated with the same letter were not significantly different at the P<0.01 level.

Table 2
Heritability values (h^2) and variance components for agronomic traits of alfalfa cultivars

Characters	h^2	Vp	Vgy	Vg	PVC	GVC
Main stem height	0.910	113.630	0.407	103.420	1.74	1.58
Main stem diameter	0.976	0.389	0.001	0.380	9.30	9.10
Leaflet length	0.818	0.044	0.0077	0.036	2.30	1.90
Leaflet width	0.888	0.0134	0.0013	0.0119	1.61	1.40
Leaf/stem ratio	0.904	0.021	0.001	0.019	1.90	1.70
Racemes/main stem	0.283	7.301	5.156	2.065	5.42	1.47
Raceme length	0.990	0.330	0.00067	0.327	8.30	8.20
Seeds/pod	0.992	1.316	0.01	1.306	2.45	2.43
1000-seeds weight	0.880	0.075	0.009	0.066	3.39	2.90
Herbage yield	0.972	225.473	5.414	219.153	4.50	3.91
Dry matter yield	0.996	9.050	0.005	9.022	5.60	5.59
Seed yield	0.954	0.0066	0.00033	0.0063	1.73	1.63

h^2: Broad sense heritability value, Vp: Phenotypic variance, Vg: Genotypic variance, Vgy: Genotype × year variance, PVC: Phenotypic variance coefficient, GVC: Genotypic variance coefficient.

Plant height, main stem diameter, stems/plant, leaves/plant, leaf length, leaflet width and length, and leaflets/leaf are important properties that are used to estimate herbage yield (Tekeli and Ates, 2003a; b). Marina exhibited higher values ($P<0.01$) than the other cultivars for the main stem height (78.69 cm), main stem diameter (4.85 mm), leaflet width (0.93 cm), number of seeds per pod (6.57), herbage yield (75.64 t ha^{-1}), dry matter yield (20.06 t ha^{-1}) and seed yield (0.49 t ha^{-1}) ($P<0.01$). Şengül and Sağsöz (1997) stated that alfalfa grew to a height of 122 cm, whereas Petkova et al. (2003) found this value to be only 49.6–64.7 cm. The stem height values recorded in the present experiment were lower than those reported by Şengül and Sağsöz (1997), but similar to those found by Petkova et al. (2003). The adaptation and certain agricultural characters of alfalfa cultivars were investigated by Dikmen (1992), who reported a maximum stem diameter of 3.15 mm, and herbage yields and hay yields ranging from 12.27–18.46 t ha^{-1} and 3.08–5.10 t ha^{-1}, respectively. Avcıoğlu et al. (1999) found a herbage yield of 12.68 t ha^{-1} and a dry matter yield of 3.82 t ha^{-1} for alfalfa. The present herbage and dry matter yields were higher than those reported by Dikmen (1992). Soya et al. (1997) reported 3–7 seeds/pod and 0.4–1.5 t ha^{-1} seed yield from alfalfa, similar to the present findings.

Leaflet length ranged from 1.65 to 2.08 cm, the greatest leaflet length being measured in Marina (2.08 cm), followed by Sitel (2.04 cm) and Elçi (1.90 cm) ($P<0.01$). The highest values for the leaf/stem ratio were determined as 1.22 and 1.19 in Marina and Bella, respectively. A lower leaf/stem ratio of 0.55–0.72 was found by Dikmen (1992).

The number of racemes per main stem, raceme length, number of seeds per pod and 1000-seed weight are important traits used to determine seed yield. There were no significant differences between the alfalfa cultivars for the

number of racemes per main stem (P>0.05, 0.01). The number of racemes per main stem ranged from 11.99 to 16.63, with a raceme length of 3.15 to 4.38 cm. Lower raceme length (1.0–2.5 cm) was reported by Soya et al. (1997). The highest 1000-seed weights (2.49 and 2.42 g) were determined for Marina and Sitel, respectively, which is in agreement with the figure of 2–3 g reported by Açıkgöz (2001).

Heritability was low for the number of racemes per main stem (28.3%) and the leaflet length (81.8%). These traits may be affected by the environment. The estimates of heritability in the broad sense were high for the other characters, indicating that these traits were controlled by genetic factors. These findings are similar to those of Orak (2000), who recorded heritability values of 87%, 79% and 70% for number of branches, pods/plant and 1000-seed weight, respectively. Tekeli and Ateş (2002a, b) investigated the heritability and variation of some yield components in Persian clover (*Trifolium resupinatum* L.) lines and found the highest broad sense heritability value for seeds/head (95.75%), while the heritability values for stem height, leaflet length, leaflet width, 1000-seed weight, herbage and seed yields were reported to be 71.14%, 93.0%, 85.0%, 86.75%, 60.99% and 95.01%, respectively.

The number of racemes per main stem exhibited great differences in the phenotypic and genotypic variance coefficients, while smaller differences were found in the phenotypic and genotypic variance coefficients for the other traits.

The phenotypic variance coefficient was found to range from 1.61–9.30, the highest phenotypic variance coefficients being determined for main stem diameter (9.30) and raceme length (8.30). The highest genotypic variance coefficients were 9.10 and 8.20, these values being found, as in the case of genotypic variance coefficients, for main stem diameter and raceme length. Traits which show a comparatively high genotypic variance coefficient may respond favourably to selection (Debnath, 1987).

Conclusions

As the results of this investigation, it was concluded that environmental fluctuations had a greater effect on the number of racemes per main stem and the leaflet length than on other characters, so these factors may be considered as practical selection criteria for improving alfalfa cultivars.

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EFFECT OF CADMIUM ON GROWTH AND OXIDATIVE METABOLISM OF FABA BEAN PLANTS

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The effect of $CdCl_2$ (0–50 μM) on the growth, physiological parameters and leaf antioxidative enzymes of faba bean plants was studied in order to investigate the possible involvement of this metal in the generation of oxidative stress. In the roots and leaves of faba bean plants Cd produced a significant inhibition of growth, as well as a reduction in the transpiration rate, photosynthetic efficiency ($^{14}CO_2$-fixation), ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco) activity and leaf pigment content, and an alteration in the nutrient status in booth roots and leaves. An increased level of free proline was also detected. The results suggest that the treatment of faba bean plants with $CdCl_2$ induced a concentration-dependent oxidative stress situation in the leaves, characterized by an accumulation of H_2O_2, as a result of the inhibition of the antioxidant enzymes glutathione reductase (GR) and catalase (CAT). These results point to the possible induction of leaf senescence by cadmium.

Key words: cadmium, catalase, faba bean, glutathione reductase, photosynthesis ($^{14}CO_2$-fixation), oxidative stress

Introduction

Vicia faba L. is considered to be one of the world's most important legume crops. In Egypt, this plant occupies an area of 105,000 ha (FAO, 1981) and represents a major crop for human food, animal feed uses and industrial purposes. Cadmium (Cd^{2+}) is a highly toxic environmental pollutant found in air, water and soil and is non-essential for plants. It which enters the environment mainly from industrial processes and phosphate fertilizers and is then transferred to the food chain (Wagner, 1993). Cd accumulation causes a reduction in photosynthesis and nutrient uptake (Sanità di Toppi and Gabbrielli, 1999), and results in visible symptoms of injury in plants, such as chlorosis, growth inhibition and finally death (Kahle, 1993), although the mechanisms involved in its toxicity are still not completely understood. Cadmium produces alterations in the functionality of membranes by inducing changes in lipid composition (Ouariti et al., 1997) and by affecting the enzymatic activities associated with membranes, such as that of H^+-ATPase (Fodor et al., 1995). The photosynthesis process is sensitive to Cd, chlorophyll being one of the targets (Somashekaraiah et al., 1992) as well as the enzymes involved in CO_2 fixation (Greger and Ögren, 1991). Cadmium toxicity is also correlated with disturbances in the uptake and distribution of macronutrients in plants (Gussarson et al., 1996). Cd produces oxidative stress, possibly by generating free radicals and active oxygen species

(Hendry et al., 1992). These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of the enzymes, thus affecting cell viability. The enzymes GR (an important component of the ascorbate–glutathione cycle) and CAT are involved in the detoxification of reactive oxygen species (ROS), thereby preventing the formation of OH radicals in various cellular compartments (Jiménez et al., 1997). In *Phaseolus vulgaris*, the toxicity of Cd has been related to alterations in the antioxidant systems (Chaoui et al., 1997). The objective of this work was to investigate whether cadmium could adversely influence the growth of Faba bean.

Materials and methods

Plant material and growth conditions

Faba bean seeds (*Vicia faba* cv. Giza 2) were purchased from the Crop Institute, Agriculture Research Center, Giza, Egypt. After surface sterilization with 0.1% $HgCl_2$ for 16 min, they were rinsed thoroughly with distilled water and germinated on moist filter paper for three days in an incubator at 25°C until their radicles emerged. The pregerminated seeds were grown in 30 cm diameter black polyethylene pots in sand culture at a day/night temperature of 24/18°C, with 70% relative humidity, 14 h light and a photon flux density of 200 μmol $m^{-2}s^{-1}$. Four seedlings were maintained per pot. The pots were irrigated with 0.25 strength of the nutrient solution reported by Yang and Shen (1996), which was gradually increased to full strength on the day of cadmium stress imposition. A 300 ml volume of the nutrient solution pot was supplied twice daily to each pot. The stress treatments were imposed abruptly twenty-five days after seedling emergence. In these treatments, the pots were irrigated with full strength nutrient solution with or without the desired concentrations of $CdCl_2$ (0.0, 10, 20, 30, 40 and 50 μM) and the plants continued to receive the appropriate solutions for 50 days. Five replicates of each treatment were prepared to give a total of 30 pots. The growth data of intact seedlings (length, fresh and dry weights of shoot and root, and leaf area/plant) were recorded.

The leaves were washed and homogenized in 50 mM Tris-HCl buffer (pH 7.5) containing 0.1 mM EDTA, 2 mM dithiothreitol, 0.2% (v/v) Triton X-100 and 1 mM phenyl methylsulphonyl fluoride (1/4, w/v). The homogenates were centrifuged at 27,000 *g* for 20 min and the supernatants were used for free proline and enzyme determinations.

Enzyme assays

CAT activity (EC 1.11.1.6) was determined as described by Aebi (1984) and GR activity (EC 1.6.4.2) was assayed as given by Jiménez et al. (1997). The activity of Rubisco was estimated according to Vu et al. (1997).

Macronutrient and cadmium determination

The samples of leaves and roots were washed and then mineralized with perchloric acid. For macronutrient determination, the samples were digested with sulphuric acid and H_2O_2. N and P were determined by colorimetry, Ca, Mg and Cd by atomic absorption spectrophotometry, and Na and K by flame photometry (AAS 6 Vario, Analytical Jena GmbH).

Scanning electron microscopy (SEM)

For SEM, the samples were fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), dehydrated in a graded ethanol series (30–100%, v/v), critical-point-dried through carbon dioxide, mounted on stubs, and coated with gold. The material was observed at 20 KV in a DM950 Carl Zeiss scanning electron microscope.

Other assays

Photosynthetic activity ($^{14}CO_2$-fixation) was measured in the Atomic Energy Authority Radioisotope Department, Cairo, Egypt, according to Moussa (2001). One pot from each treatment was placed under a Bell jar, which was used as a photosynthetic chamber. Radioactive $^{14}CO_2$ was generated inside the chamber by a reaction between 10% HCl and 50 μCi (1.87×10^6 Bq) $NaH^{14}CO_3$ + 100 mg Na_2CO_3 as carrier. Then the samples were illuminated with a tungsten lamp. After 30 min exposure time, the leaves were quickly detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction by 80% hot ethanol. The ^{14}C was assayed from the ethanolic extracts in soluble compounds using a Bray Cocktail (Bray, 1960) and a Liquid Scintillation Counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises). The transpiration rate was determined as described by Ludlow and Muchow (1990), the free proline content as described by Bates et al. (1973) and the pigment contents by the method of Inskeep and Bloom (1985). The data were analysed statistically and mean values were compared using Duncan's New Multiple Range Test according to Steel and Torrie (1984).

Results

Effect of Cd treatment on plant growth

Increasing concentrations of Cd in the nutrient solution produced a significant decrease in the leaf area/plant, shoot and root lengths, and fresh and dry weights of faba bean plants (Table 1). The effect of higher Cd concentrations ranging between 75 and 150 μM was also studied, but the plants were considerably damaged by 70 μM (data not shown), so the study focused on 50 μM as the highest Cd concentration. The application of $CdCl_2$ (10 and 50 μM) caused a 3.6% and 56.5% inhibition in the shoot length and an 11.6% and 78.2% reduction in the root length, respectively, as compared with the untreated samples (Table 1). $CdCl_2$ (10 and 50 μM) treatment induced a great reduction in the shoot fresh weight (4.9% and 62.8%) and shoot dry weight (6.9% and 56.5%) of faba bean plants compared with the control samples. The application of $CdCl_2$ (10 and 50 μM) to faba bean plants reduced the root fresh weight by 11.6% and 73.7% and the root dry weight by 18.4 and 80.7% compared with the control plants. In response to treatments with $CdCl_2$ (10 and 50 μM), faba bean plants exhibited considerable decreases in their leaf area/plant (11.2% and 70.5%) in comparison with the control plants (Table 1).

The Chl *a/b* ratio decreased with increasing Cd concentrations (Table 2). The growth inhibition of faba bean plants treated with $CdCl_2$ (10 and 50 μM) was accompanied by a significant decrease of 30.5% and 85.6% in photosynthetic activity, in comparison with the control plants (Table 2). The transpiration rate was also affected by $CdCl_2$ treatment (10 and 50 μM), exhibiting a significant decrease of 14.9% and 52.9% with respect to the control samples (Table 2).

Table 1
Effect of Cd treatment on the growth characteristics of faba bean plants

Cd (μM)	Length (cm)		Fresh weight (g)		Dry weight (g)		LA*/plant (cm^2)
	Shoot	Root	Shoot	Root	Shoot	Root	
0.0	58.9^a	14.7^a	26.13^a	6.95^a	4.21^a	1.14^a	50.86^a
10	56.8^a	13.0^a	24.85^b	6.14^a	3.92^b	0.93^a	45.16^b
20	52.3^b	10.1^b	21.61^c	5.26^b	3.34^c	0.71^b	36.91^c
30	45.1^c	6.9^c	16.26^d	3.97^c	2.81^d	0.53^c	31.82^d
40	37.4^d	5.2^d	14.02^e	3.12^d	2.53^d	0.39^d	27.91^e
50	25.6^e	3.2^e	9.73^f	1.83^e	1.83^f	0.22^e	15.01^f

*LA: Leaf area; Values are means of three replicates. Values followed by the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test

Table 2
Effect of Cd treatment on pigment contents, photosynthesis and transpiration rate of faba bean plants

Cd (μM)	Pigment contents (mg g^{-1} FW)				Photosynthetic activity (*dpm mg^{-1} FW)	Transpiration rate (mM H_2O $m^{-2}s^{-1}$)
	Chl *a*	Chl *b*	Carotenoids	*a/b* ratio		
0.0	6.88^a	3.85^a	1.45^a	1.88	14846^a	3.02^a
10	6.32^a	3.91^a	1.43^a	1.62	10178^b	2.57^b
20	5.02^b	3.35^b	1.16^b	1.50	5359^c	2.15^c
30	4.13^c	3.06^c	1.05^b	1.35	6039^d	1.97^d
40	2.95^d	2.51^d	0.82^c	1.17	4868^e	1.99^e
50	1.47^e	1.81^e	0.31^d	0.81	2108^f	1.42^f

*Disintegration per minute; Values are means of five replicates. Values followed by the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Ultrastructural studies

SEM analysis of the abaxial side of faba bean leaves showed stomatal closure in plants treated with 50 μM Cd, while in the control plants most stomata were open. Cd induced an increase in the leaf cells compared to the control sample (Fig. 1).

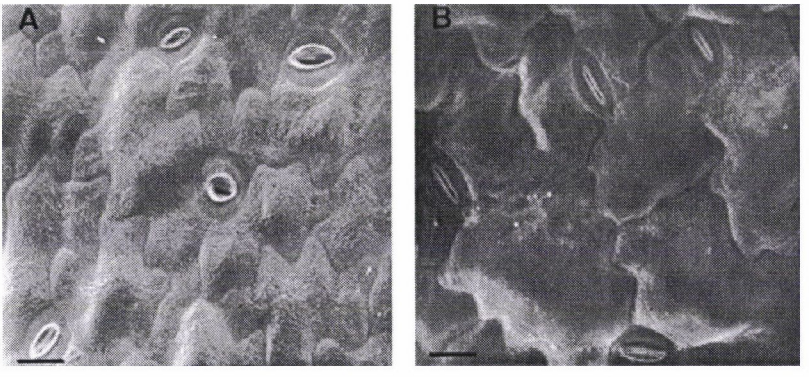

Fig. 1. Scanning electron microscopy micrographs showing the effect of Cd on stomatal closure in faba bean leaf cells. (A) Control leaf. (B) Leaf from 50 μM Cd-treated plants. Bar represents 10 μm

Concentrations of macroelements and cadmium

Cd altered the content of macronutrients in both the leaves and roots (Table 3). The Ca content was significantly reduced in the leaves, but did not show a significant change in the roots, while the content of Mg was significantly reduced in both roots and leaves. The N content was diminished by Cd treatment in the leaves but in the roots a slight increase was detected. The contents of P and K showed a significant reduction after Cd treatment in the leaves, while in the roots the decreases in both macronutrients were only significant at 40 and 50 μM Cd (Table 3). Cadmium was mainly accumulated in the roots, followed by the leaves, although the capacity to accumulate this metal increased at increasing Cd concentrations in the nutrient solution (Table 3).

Effect of Cd on the activities of GR, CAT and Rubisco

In the leaves, the activity of CAT, GR and Rubisco was depressed with increasing concentrations of Cd. The free proline contents also showed a significant increase as the result of cadmium treatment (Table 4). In faba bean plants grown at 50 μM $CdCl_2$ there was a great inhibition in the GR (53.2%), CAT (73.5%) and Rubisco (71.7%) activity as compared with that in the control plants (Table 4). $CdCl_2$ treatment at 10 and 50 μM also significantly increased the proline content of faba bean plants by 17% and 183%, respectively, compared with that of the control plants (Table 4).

Table 3

Effect of Cd treatment on macronutrients (mg g^{-1} DW) and Cd content (μg g^{-1} DW) in leaves and roots of faba bean plants

Cd (μM)	Leaves						Roots					
	N	P	K	Ca	Mg	Cd	N	P	K	Ca	Mg	Cd
0.0	683^{a}	60^{a}	529^{a}	368^{a}	65^{a}	1.5^{c}	234^{c}	65^{abc}	691^{ab}	170^{ab}	83^{a}	4.2^{d}
10	445^{b}	43^{b}	510^{a}	227^{b}	60^{ab}	48.9^{bc}	335^{ab}	67^{ab}	625^{ab}	178^{a}	48^{bc}	5131^{c}
20	385^{bc}	26^{c}	370^{b}	205^{bc}	50^{bc}	69.3^{b}	381^{a}	82^{a}	603^{a}	190^{a}	51^{abc}	9428^{b}
30	341^{bc}	30^{bc}	361^{a}	165^{cd}	41^{c}	81.6^{b}	368^{a}	70^{ab}	528^{ab}	165^{ab}	63^{ab}	14912^{a}
40	301^{cd}	33^{bc}	265^{c}	170^{cd}	43^{c}	99.5^{b}	289^{bc}	54^{bc}	470^{a}	125^{ab}	39^{bc}	16873^{a}
50	209^{d}	21^{c}	198^{c}	120^{d}	30^{d}	168.7^{a}	236^{bc}	45^{c}	382^{c}	98^{b}	24^{c}	17424^{a}

Values are means of five replicates. Values followed by the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4

Effect of Cd treatment on the catalase (μmol H_2O_2 min^{-1} mg^{-1} protein), glutathione reductase (nmol NADPH min^{-1} mg^{-1} protein), Rubisco (*nKat g^{-1} FW) and free proline (μg g^{-1} FW)content of faba bean leaf crude extracts

Parameter	Cd (μM)					
	0.0	10	20	30	40	50
GR	410^{a}	375^{b}	338^{c}	290^{d}	243^{e}	192^{f}
CAT	83^{a}	88^{b}	71^{c}	55^{d}	40^{e}	22^{e}
Rubisco	46^{a}	44^{a}	35^{b}	26^{c}	20^{d}	13^{d}
Free proline	53^{a}	62^{b}	78^{c}	91^{d}	119^{e}	150^{f}

*Mol/sec × 10^{-9}; Values are means of 10 replicates. Values followed by the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test

Discussion

The aim of this work was to evaluate the effect of Cd on the elongation growth, biomass production and enzymatic or non-enzymatic ROS scavenging mechanisms of *Vicia faba* plants. Cadmium produced a significant reduction in the growth of faba bean plants, as also observed by Metwally et al. (2003) and Dixit et al. (2001). The difference in the volume of epidermal cells in control and treated plants might be due to the action of Cd on the mitotic division of epidermal cells (Zhang and Yang, 1994), leading to a reduction in the surface area of the treated plant leaf (No. of epidermal cells/unit area). To alleviate this reduction, the volume of cells in treated plants was greater as compared with control plants. These results are in accordance with data in the literature (Rosko and Rachlin, 1977; Barceló et al., 1988; Baryla et al., 2001). The growth inhibition produced by Cd could be due mainly to the effect of this heavy metal on growth and on the photosynthesis rate (Metwally et al., 2003). Cd causes a degradation of chlorophyll and carotenoids as well as an inhibition of their biosynthesis (Somashekaraiah et al., 1992; Küpper et al., 1998), which could result in disturbances in the electron transport rates of PSI and PSII, leading to the generation of oxygen free radicals. Rubisco is the primary enzyme of photosynthetic carbon fixation. Cd^{2+}, as a divalent cation, may displace the Mg^{2+} ions which act as activators for Rubisco, resulting in a loss of activity (Wildner and Henkel, 1979). The highest Cd concentration decreased the Rubisco regeneration capacity of the Calvin cycle and photosynthesis (Pankovic et al., 2000). Cd caused a decrease in the transpiration rate of *Picea abies* (Schlegel et al., 1987). The present results suggested that the adverse effects of Cd on photosynthetic efficiency could be attributed to its inhibitory action on Rubisco activity and pigment content and to the limitation of CO_2 assimilation due to stomatal closure, which was paralleled by a decreased transpiration rate.

In general, the concentration of macronutrients was severely reduced in the leaves, while in the roots the changes were only significant at 40 and 50 μM Cd. Similar results have been observed in barley (Metwally et al., 2003). The accumulation of K can also be affected by the Cd-dependent modification of ATPases, as described for birch (Gussarson et al, 1996). As the roots are non-photosynthetic tissues the flux of ROS is presumably low. Despite the higher accumulation in the roots, the level of free Cd ions in the roots may remain low, since most of the Cd ions are either immobilized or compartmentalized in vacuoles or form Cd-phytochelatin complexes, while Cd accumulation in the leaves is relatively low due to transport barriers and may be a strategy to protect photosynthetic functions from Cd-induced oxidative stress (Dixit et al., 2001).

GR activity was reduced at higher Cd concentrations (Schickler and Caspi, 1999). The decline of CAT activity has been associated with Cd toxicity in *Phaseolus vulgaris* (Chaoui et al., 1997).

At $CdCl_2$ concentrations of 40 and 50 μM the free proline content increased (Metwalley et al., 2003). It has been suggested that free proline acts as an osmoprotectant (Delauney and Verma, 1993) and as a metal chelator (Farago and Mullen, 1979). Proline also acts directly as an antioxidant to protect the cell from free radical damage and maintain a more reducing environment that is favourable for phytochelation synthesis and Cd sequestration (Surasak et al., 2002).

Oxidative deterioration is considered to be an intrinsic feature of the senescence process of leaves (del Río et al., 1998). Senescence is also characterized by a cessation of photosynthesis, as well as of other processes (Buchanan-Wollaston, 1997). All these symptoms were observed in the present work on faba bean plants, the most important being photosynthetic activity, which was reduced about seven times at 50 μM $CdCl_2$, in comparison with the control plants, and may suggest that Cd toxicity could induce leaf senescence.

In summary, it was concluded that the Cd treatment of faba bean plants could retard their growth via an inhibition of associated biochemical processes, e.g. enzyme activity, photosynthesis and the macronutrient content.

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VARIABILITY IN THE RESPONSE OF PEARL MILLET [*PENNISETUM AMERICANUM* (L.) LEEKE] ACCESSIONS TO SALINITY

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The objective of this project was to develop understanding about the possibility of improving salt tolerance in pearl millet using selection and breeding methods. A collection of 143 pearl millet accessions was obtained from nineteen countries in different regions of the world, mostly from dry hot environments, e.g. Yemen, Sudan, the Central African Republic and Niger. Considerable genetic variability was found in these accessions for salt tolerance. Based upon a preliminary examination of the responses to NaCl solution in a selection of accessions, it was decided that 160 mM NaCl would be the reference parameter for assessing tolerance. The six most salt-tolerant accessions were 10876 and 10878 from Sudan, 18406 and 18570 from Namibia, and ICMV-93753 and ICMV-94474 from India, all of which had relative root lengths of above 70%. Accessions 213011 and 21351 were very sensitive, their relative root length being below 30%. Unfortunately, the areas from which the tolerant accessions from Sudan, Namibia and India originated are not known, but it is possible that they may have inhabited dry, saline lands.

Key words: genetic diversity, pearl millet, salinity, sodium chloride, variability, stress tolerance

Introduction

In the wild, natural selection has been promoting adaptation to changing environments throughout time in plants, animals, and all other living organisms. Since plants have been grown and animals exploited by man, artificial selection has transformed these organisms, as well as natural selection. This has been the product of two components, selection, and the genetically based diversity/variation in the organisms being selected, a component existing in all living creatures.

Selecting for salinity tolerance is very difficult under field conditions, because salt-affected regions are typically very patchy in position and salinity, and any field will contain areas where salinity is absent as well as areas where salinity is very high, in which even the most tolerant halophytes will not grow (Hajrasuliha et al., 1980; Richards, 1983). This patchiness may occur over distances of less than a metre or over much larger distances. To carry out experiments in the "open field" for assessing salinity tolerance in crop species is

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almost impossible, because there is substantial variation in the salinity depth in the soil profiles, and over time. It is therefore difficult to determine the degree of salinity in saline soils, because selection and evaluation in the field are ineffective. Because of patchiness, and because of the ease with which plants can be grown in uniform salinized nutrient solutions, many selection and genetic studies on salt tolerance have been made in nutrient solution under controlled conditions.

Considerable variation for salt tolerance has been reported within cultivars, land races and lines within a number of crops, e.g. wheat (Martin et al., 1994; Akram et al., 2002), lucerne (Al-Khatib et al., 1993), barley (Ahmad et al., 2003), sorghum (Azhar and McNeilly, 1987), lentil (Ashraf and Waheed, 1990), maize (Maiti et al., 1996), spinach (Wilson et al., 2000), rice (Aslam et al., 1996) and pearl millet (Kebebew and McNeilly, 1995) and all suggested that selection for increased tolerance to salinity in pearl millet should be possible.

Pearl millet is the sixth most important of the world cereals, widely cultivated in semi-arid tropics as a major staple food crop. It is grown on an estimated 28 million hectares of the world (Choi et al., 1997). The grain is used to make bread in south Asia or prepared as gruel, couscous and beer in Africa, and is also used as animal feed and forage. It is the principal grain crop in areas too hot and dry for other cereals.

It may be possible to produce conventional crops that can adapt and give a reasonable yield under saline conditions provided the required genes are present in the crop. Genetic variability for salinity tolerance is the primary prerequisite for any breeding programme. Considering the rate of increase in the area of land suffering from soil salinity, it is very important to launch extensive research projects to develop salt tolerant lines/varieties of each crop, which may be used on salt-affected soils without the requirement of substantial financial support for soil reclamation or management.

Very limited information is available on pearl millet, a crop that could be exploited to improve salinity tolerance. In order to provide a broader basis for developing salinity-tolerant lines of *Pennisetum americanum*, a greater amount of material must be examined. This paper describes experiments on the variability in 143 millet accessions of diverse origin using the methods followed by Ashraf and McNeilly (1992).

Materials and methods

Plant material

Pearl millet (*P. americanum*) accessions were obtained from gene bank sources and institutes in various countries. The data of 143 accessions, varieties and land races obtained from various arid or semi-arid parts of the world are recorded in Table 1.

Table 1
Absolute and relative root length of different pearl millet accessions (with country of origin) in 160 mM NaCl

Serial No.	Accession No.	Country of origin	Control root length (cm)	Root length in 160 mM NaCl	Relative root length (%)
1	164410	India	10.44	4.31	41.30 II
2	164421	India	15.24	4.37	28.70 III
3	185642	Ghana	18.90	8.26	43.70 II
4	186338	Australia	17.60	6.09	34.61 III
5	213011	India	12.51	2.71	21.66 III
6	213531	India	14.55	4.10	28.15 III
7	214329	India	16.03	5.31	33.14 III
8	215602	India	15.45	5.36	34.72 III
9	217952	Pakistan	15.54	8.06	51.83 II
10	286850	Nigeria	18.10	9.71	53.67 II
11	286855	Nigeria	15.37	9.78	63.68 II
12	286872	Nigeria	15.10	8.91	59.01 II
13	286874	Nigeria	14.09	6.71	47.62 II
14	286901	Nigeria	14.20	7.38	51.98 II
15	286917	Nigeria	19.17	8.61	44.93 II
16	286919	Nigeria	17.07	11.97	70.12 I
17	286937	Nigeria	18.61	9.09	48.86 II
18	286967	Nigeria	20.27	8.45	41.67 II
19	286972	Nigeria	15.89	7.98	50.23 II
20	287003	Nigeria	15.65	5.08	32.46 III
21	287014	Nigeria	13.72	7.76	56.56 II
22	287019	Nigeria	16.09	9.41	58.47 II
23	287020	Nigeria	14.64	7.21	49.24 II
24	287070	Nigeria	14.20	8.13	57.30 II
25	287097	Nigeria	13.74	5.88	42.78 II
26	296377	S. America	17.09	7.68	44.95 II
27	331353	Uganda	14.59	7.26	49.74 II
28	452269	S. Union	14.07	6.99	49.69 II
29	02-82	Tunisia	11.49	5.58	48.54 II
30	04-82	Tunisia	13.33	6.74	50.59 II
31	08-82	Tunisia	14.54	6.37	43.77 II
32	10-82	Tunisia	15.22	6.41	42.10 II
33	12-82	Tunisia	14.22	5.29	37.20 III
34	15-82	Tunisia	13.41	6.56	48.90 II
35	17-82	Tunisia	14.80	5.77	38.96 III
36	18-82	Tunisia	8.25	1.88	22.74 III
37	20-82	Tunisia	5.50	2.50	50.00 II
38	24-82	Tunisia	15.27	4.38	28.70 III
39	PEN 678/85	Libya	11.13	6.94	62.38 II
40	PEN 837/93	Tunisia	13.74	7.21	52.49 II
41	PEN 558/93	Israel	10.82	4.47	41.26 II
42	25113	Yemen	17.97	7.43	41.34 II
43	25136	Yemen	19.18	9.01	46.99 II
44	25142	Yemen	12.99	6.76	52.02 II
45	25149	Yemen	17.43	7.11	40.79 II

Table 1 continued

46	25160	Yemen	19.95	7.76	38.89 III
47	25233	Yemen	17.77	5.79	32.59 III
48	25237	Yemen	21.26	7.38	34.71 III
49	25414	Yemen	16.64	8.26	49.60 II
50	25440	Yemen	19.67	7.04	35.76 III
51	25474	Yemen	22.65	9.44	41.69 II
52	25477	Yemen	19.55	6.47	33.11 III
53	25488	Yemen	15.48	7.30	47.20 II
54	25491	Yemen	19.21	8.29	43.15 II
55	25493	Yemen	16.70	7.68	45.99 II
56	25496	Yemen	18.52	7.69	41.53 II
57	25514	Yemen	16.21	8.24	50.82 II
58	25516	Yemen	19.68	8.23	41.84 II
59	25527	Yemen	19.29	9.09	47.11 II
60	25549	Yemen	17.28	7.21	41.74 II
61	25560	Yemen	18.05	6.73	37.29 III
62	25613	Yemen	15.96	8.19	51.32 II
63	26102	Yemen	16.79	7.06	42.02 II
64	286838	Nigeria	14.76	6.39	43.32 II
65	286893	Nigeria	14.63	7.64	52.24 II
66	286894	Nigeria	15.27	6.61	43.31 II
67	286908	Nigeria	14.45	7.46	51.60 II
68	286910	Nigeria	16.55	8.46	51.13 II
69	286911	Nigeria	20.14	7.00	34.77 III
70	286975	Nigeria	14.94	5.19	34.71 III
71	286976	Nigeria	13.08	5.15	39.39 III
72	286994	Nigeria	16.14	8.05	49.87 II
73	5906	Senegal	10.14	4.85	47.87 II
74	5960	Senegal	10.15	3.45	34.02 III
75	5995	Senegal	12.85	5.30	41.25 II
76	6021	Senegal	9.67	4.99	51.57 II
77	6031	Senegal	10.53	5.01	47.55 II
78	10495	Senegal	12.49	4.91	39.32 III
79	10501	Senegal	9.46	4.23	44.67 II
80	10510	Senegal	9.45	4.55	48.20 II
81	10525	Senegal	12.40	6.32	50.94 II
82	10528	Senegal	6.37	5.08	79.86 I
83	10866	Sudan	7.54	5.02	66.54 II
84	10876	Sudan	5.87	5.08	86.47 I
85	10878	Sudan	5.92	5.31	89.59 I
86	10905	Sudan	9.66	5.29	54.74 II
87	10910	Sudan	8.58	5.15	60.06 II
88	17407	C. African Rep.	19.38	10.92	56.35 II
89	17410	C. African Rep	17.27	9.68	56.07 II
90	17411	C. African Rep	16.49	8.46	51.33 II
91	17415	C. African Rep	18.75	9.06	48.30 II
92	17418	C. African Rep	16.77	11.14	66.44 II
93	18205	India	15.58	8.63	55.42 II
94	18261	India	14.89	8.02	53.87 II
95	18278	India	17.77	6.21	34.93 III

Table 1 continued

96	18309	India	10.49	5.85	55.79 II
97	18319	India	14.75	6.76	45.85 II
98	18365	Namibia	13.97	9.00	64.44 II
99	18406	Namibia	12.77	10.01	78.38 I
100	18570	Namibia	14.20	9.88	69.58 I
101	18708	Namibia	13.46	9.03	67.06 II
102	18709	Namibia	16.32	10.07	61.68 II
103	20595	Nigeria	16.05	6.93	43.20 II
104	20597	Nigeria	16.26	7.84	48.20 II
105	20665	Nigeria	17.24	9.74	56.51 II
106	20690	Niger	17.44	6.92	39.68 III
107	20702	Niger	17.38	5.91	34.00 III
108	20705	Niger	17.90	7.26	40.54 II
109	20720	Niger	17.95	9.67	53.86 II
110	20725	Nigeria	17.37	10.24	58.96 II
111	20728	Nigeria	15.78	6.19	39.25 III
112	20735	Nigeria	16.98	8.19	48.21 II
113	PARC-MS-1	Pakistan	18.39	9.95	54.10 II
114	PARC-MS-2	Pakistan	17.71	9.71	54.86 II
115	DB-V	Pakistan	19.03	9.92	52.14 II
116	MP-771	India	18.21	12.04	66.08 II
117	WC-C-75	India	18.96	9.65	50.92 II
118	DBR-III	Pakistan	20.50	9.50	46.34 II
119	IC-8206	India	21.00	10.65	50.71 II
120	Y-72	Pakistan	21.30	9.95	46.70 II
121	Y-84	Pakistan	16.54	10.91	65.98 II
122	B-18	Pakistan	23.08	10.21	44.22 II
123	ICMV-31293	ICRISAT India	18.07	11.92	65.98 II
124	ICMV-93753	ICRISAT India	15.17	12.82	84.49 I
125	ICMV-94132	ICRISAT India	15.06	10.15	67.40 II
126	ICMV-94474	ICRISAT India	16.62	12.04	72.44 I
127	ICMV-94475	ICRISAT India	15.83	10.21	64.51 II
128	ICMV-95105	ICRISAT India	16.51	9.48	57.41 II
129	ICMV-95107	ICRISAT India	15.43	10.15	65.77 II
130	ICMV-95777	ICRISAT India	17.90	8.65	48.34 II
131	93607	Italy	12.19	5.46	44.77 II
132	93608	Italy	9.92	4.47	45.04 II
133	93610	Italy	12.75	6.04	47.32 II
134	93612	Italy	21.34	8.24	38.61 III
135	93613	Italy	14.18	6.10	43.03 II
136	93615	Italy	16.33	6.62	40.52 II
137	93616	Italy	11.96	5.41	45.24 II
138	2576	Ethiopia	14.10	9.72	68.95 II
139	2579	Ethiopia	16.47	8.02	48.69 II
140	2785	Ethiopia	17.29	7.56	43.75 II
141	89004	Ethiopia	17.77	7.16	40.30 III
142	Kaufela	Ethiopia	16.08	9.45	58.74 II
143	Hoon	Ethiopia	13.48	6.77	50.22 II

0–40% Sensitive (III); 41–69% Moderately tolerant (II); 70–100% Tolerant (I)

Testing of pearl millet germplasm for sodium chloride tolerance

The 143 accessions were screened for salinity tolerance in a growth room maintained at 24 ± 1°C, relative humidity 60–70% and a daylength of 16 h at an intensity of 27 Wm^2. The pots were enclosed within Perspex chambers having small holes to control the degree of solution evaporation. The seedlings were grown for 14 days in the culture solution using Rorison's solution (Hewitt, 1966) and the techniques described by Ashraf et al. (1986). The concentration used for assessment was chosen from the prior growth of a random selection of six accessions, grown on seven NaCl concentrations, namely 0, 100, 115, 130, 145, 160 and 175 mM, to find the most effective concentration for distinguishing tolerant and non-tolerant accessions (Fig. 1). Twenty seeds from each accession were sown on rafts of black alkathene beads, five layers deep, floating on nutrient solution containing the required NaCl concentration in 200 cm^3 plastic beakers. Each experiment was replicated three times. Before planting, the seeds were surface sterilised in 5% sodium hypochloride solution for 5–10 minutes. Each experiment was set up as a completely randomised design. After 14 days, ten randomly chosen seedlings from each replicate were measured for root length, from which the relative tolerance was determined.

Data analysis

The data were subjected to analysis of variance, using MANOVA (SPSS, 1994). Broad sense heritability (h^2_B) was estimated as:

$$h^2_B = V_G / V_P$$

where V_G is genotypic variance and V_P phenotypic variance (Falconer and Mackay, 1996).

Results

Test experiment

The root length decreased significantly as the salt concentration increased, but that of three accessions, 2576, 2579 and Kaufela (Fig. 1) increased up to 100 mM, and then continuously decreased. Accession 2576 was the most tolerant, having the highest relative root length of 69% at 160 mM NaCl, when Kaufela and Hoon had root lengths of 59% and 50%, while at 175 mM NaCl Kaufela (51%) and Hoon (45%) had the highest root lengths. Accession 89004 was the most sensitive, exhibiting the lowest (33%) root length. Therefore, the 160 mM NaCl concentration was chosen to distinguish the extent of variability among the 143 accessions.

Relative root length

Table 1 demonstrates the origins of the accessions, the mean root length values under control and stress (160 mM NaCl) conditions, and the relative salinity tolerance values. The relative tolerance of the accessions is shown on an arbitrary scale, where III = 0 to 40%, sensitive accessions, II = 41 to 69%, moderately tolerant accessions, and I = above 70%, tolerant, potentially useful for breeding to improve tolerance. From the 143 genotypes assessed, only eight accessions, 286919 (70%), 10528 (80%), 10876 (86%), 10878 (90%), 18406 (78%), 18570 (70%), ICMV-93753 (84%) and ICMV-94474 (72%) were consistently ranked in group I. In contrast, 26 accessions were highly sensitive, with less than 40% relative root length at 160 mM. The remaining accessions were ranked as moderately tolerant.

The absolute root length of the accessions varied from 1.9 to 12.8 cm at 160 mM NaCl, and from 5.9 to 23 cm under control conditions. The effect of salinity on the root length is illustrated in Figure 2 for four tolerant, four moderately tolerant and four susceptible accessions, showing the clear difference between tolerant and susceptible accessions.

To assess the potential for exploiting the variability in the millet accessions, based on the examination of root length at increasing salt concentrations, the broad sense heritability was estimated from the analysis of variance (Table 2). A reasonable proportion of the difference in root length under saline conditions was found to be genetically based, and the heritability (h^2_B) values in the control solution and at a concentration of 160 mM NaCl were 0.91 and 0.90, respectively.

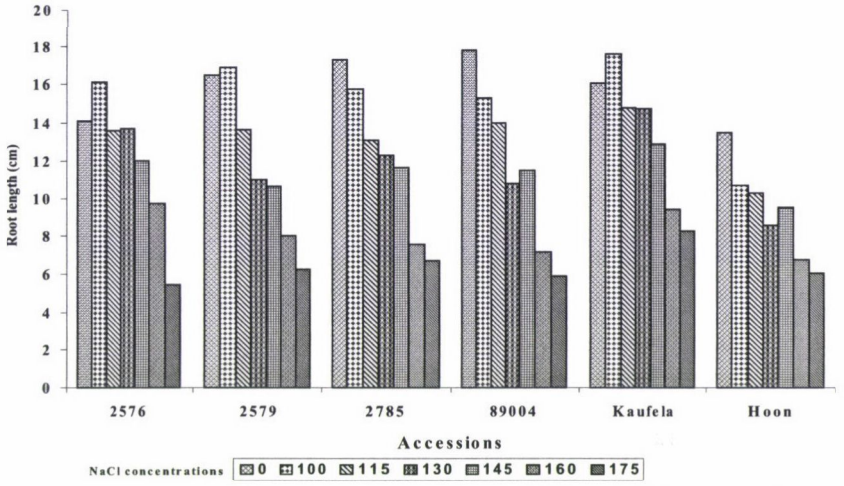

Fig. 1. Response of six pearl millet accessions at different NaCl concentrations

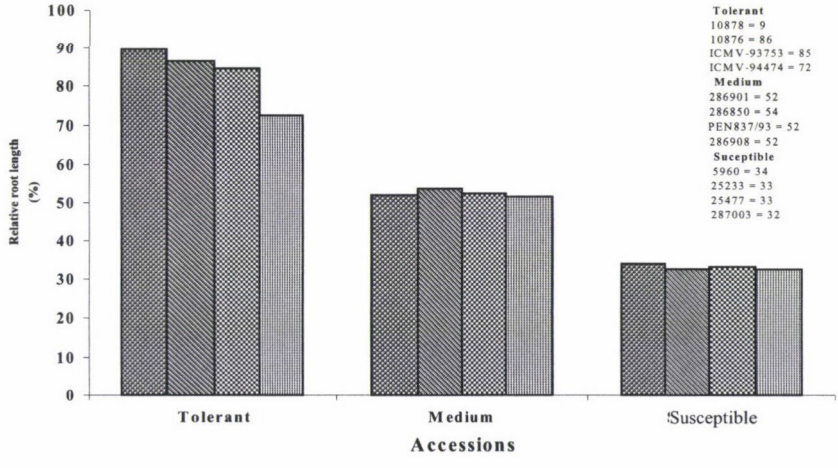

Fig. 2. Response of twelve pearl millet accessions at 160 mM NaCl concentration

Discussion

Salts affect plant growth and may even cause death due to disturbances in the soil water potential, which induces water stress in the plants and may give rise to toxic effects. Plant responses to salt stress are well documented in the scientific literature. The most relevant effect of salinity on the plant is considered to be growth retardation (Bernstein, 1975) and root growth has long been used as the first indicator of salinity effects (Flowers et al., 1977; Wyn Jones and Gorham, 1986). Breeding and selection techniques have also been used to increase plant salinity tolerance (Ashraf and McNeilly, 1992).

Root growth as an indicator of the complex characteristics determining salt tolerance is especially useful in the first steps of screening programmes (Kik, 1989). The inhibition of root growth adversely affects the survival and productivity of the plants because the roots are more sensitive to salinity than other plant components and come into contact with the solution before other plant parts (Abdul-Halim et al., 1988).

The presence of considerable genetic variability for salt tolerance in the material examined (Table 1) may be because the material used was of different origin, coming from various arid regions of the world, and originating in different sets of environments. Only 27 accessions were very sensitive to NaCl, having relative root lengths of below 30%, while there was only a small number (eight) of tolerant accessions with a relative root length greater than 70%. These results were in accordance with those of Martin et al. (1994), who screened 711 land race accessions of wheat from a broad range of countries (principally from Nepal and Pakistan) in 260 mM NaCl. Of the 367 collected from Nepal only 13 accessions survived, while none of the 80 or so accessions from Kenya survived. Two from Pakistan had a survival rate of less than 1%, and only one out of 153 from Ethiopia survived. These differences may indicate that tolerant plants originate from saline or dry regions, whilst in other regions the plants are not exposed to saline soils.

From the accessions examined it can be seen that there is no consistent relationship between the root length of seedlings grown in non-saline control solution, and the same material grown in saline solution, suggesting that each accession has a different tolerance level, making relative measurements important for assessing (Shannon, 1984). Similar information has been reported for the stress tolerance of different crop species, especially from salinity studies, e.g. on sorghum (Maiti et al., 1994), maize (Maiti et al., 1996) and lucerne (Al-Khatib et al., 1994). Data on the relative tolerance of the seedlings, based upon the root length after 14 days of growth (Table 1) show a decrease in all the accessions at 160 mM NaCl, and considerable variation between the accessions. Relative tolerance at 160 mM NaCl concentration is considered as a reference parameter for tolerance, and only accessions 10876 (87%) and 10878 (90%) from Sudan, 18406 (78%) and 18570 (70%) from Namibia, and ICMV-93753 (85%)

Table 2
Data on the genotypic variance (V_G), phenotypic variance (V_P) and broad sense heritability (h^2_B) of NaCl tolerance in pearl millet seedlings at 160 mM NaCl concentration

Component	Control	160 mM
V_G	10.75	4.32
V_P	11.80	4.77
h^2_B	0.91	0.90

and ICMV-94474 (72%) from India proved to be salt-tolerant (Table 1). This variability in the material suggests that different genetic resources and genetic material may be present in different parts of the world, and could be selected and utilised in breeding for salt tolerance. Seed material from dry and very dry countries might have NaCl tolerance. It must be remembered, however, that only a very small number of tolerant accessions were available from each country. From these results, it is clear that there are differences between the accessions, and that selection for an increase in salt tolerance could be of value.

The present results are in agreement with the findings of Ashraf and McNeilly (1992) and Kebebew and McNeilly (1995), who found considerable genetic variation in pearl millet and suggested that the improvement of salt tolerance could be attained by selection and breeding methods. Among the 143 accessions examined, eight accessions were found to be reasonably tolerant and could be useful for breeding for increased salinity tolerance. The presence of high genetic variability, coupled with high heritability, indicates that the selection of accessions with long roots under salt stress conditions could be used as a preliminary procedure in salinity breeding. The presence of continuous variation in root length under salt stress suggests that root length and salt tolerance are quantitative characters, under the control of multigenes or QTLs (quantitative trait loci).

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LEAF GROWTH AND K^+/Na^+ RATIO AS AN INDICATION OF THE SALT TOLERANCE OF THREE SORGHUM CULTIVARS GROWN UNDER SALINITY STRESS AND IAA TREATMENT

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The salt tolerance of three sorghum (*Sorghum bicolor* L.) cultivars (Dorado, Hagen Shandawil and Giza 113) and their responses to shoot spraying with 25 ppm IAA were studied. Salinity stress induced substantial differences between the three sorghum cultivars in the leaf area, dry mass, relative water content and tolerance index of the leaves. Dorado and Hagen Shandawil tolerated salinity up to 88 and 44 mM NaCl, respectively, but above this level, and at all salinity levels in Giza 113, a significant reduction in these parameters was recorded. The rate of reduction was lower in Dorado than in Hagen Shandawil and Giza 113, allowing the sequence Dorado > Hagen Shandawil > Giza 113 to be established for the tolerance of these cultivars to salinity. The differences in the tolerance of the sorghum cultivars were associated with large differences in K^+ rather than in Na^+, which was found to be similar in the whole plant. The youngest leaf was able to maintain a higher K^+ content than the oldest leaf. Consequently the K^+/Na^+ ratios were higher in the most salt-tolerant cultivar Dorado than in the other sorghum cultivars, and in the youngest than in the oldest leaf. In conformity with this mechanism, the stimulatory effect of the exogenous application of IAA was mostly associated with a higher K^+/Na^+ ratio.

Shoot spraying with IAA partially alleviated the inhibitory effect of salinity on leaf growth and on the K^+ and Ca^{2+} contents, especially at low and moderate levels of salinity, while it markedly retarded the accumulation of Na^+ in the different organs of sorghum cultivars.

Abbreviations: LA: Leaf area, DM: Dry mass, IAA: Indole acetic acid, RWC: Relative water content, TI: Tolerance index

Key words: leaf area, mineral content, relative water content, sorghum cultivars, tolerance index

Introduction

Salinity is a very important factor in sorghum yields. Differences in the salt tolerance of sorghum genotypes were observed by Maiti et al. (1994) and De La Rosa-Ibarra and Maiti (1995). Breeding for tolerance to salinity in crops has usually been limited by the lack of reliable traits for selection (Noble and Rogers, 1992). It is necessary to determine differences in resistance mechanisms between the genotypes and to incorporate characters that improve tolerance into reasonably high-yielding backgrounds.

Salinity reduces leaf growth and shortens the period of rapid leaf elongation, thereby producing shorter leaves (Bernstein et al., 1993). Salinity may cause a decrease in biomass production because increased soil salinity produces a lowering of plant water potentials, specific ion toxicities or ionic

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imbalances (Neumann, 1997). Changes in the concentrations of ions like Na^+, K^+ and Ca^{2+} may play a role in the salt tolerance of plant species. A salinity-induced increase in plant Na^+ content was associated with a decrease in K^+ and Ca^{2+} content (Serrano and Gaxiola, 1994). Therefore, the capacity of plants to maintain sufficient Ca^{2+} and K^+ may be an important factor providing a degree of salt tolerance in plants. In many glycophytes, the maintenance of a higher K^+/Na^+ ratio in the shoots, by excluding Na^+ and accumulating K^+, is associated with salt tolerance (Gorham, 1992). The mechanisms that lead to selective K^+ transfer from roots to shoots are major factors in avoiding Na^+ accumulation in the leaves (Jeschke, 1984). In upland cotton seedlings, the uptake and effective compartmentation of Na^+ in the shoots provided growth advantages over avoidance mechanisms (Leidi and Saiz, 1997).

Salinity stress could restrict the synthesis of or cause changes in plant growth promoters and increase the production of inhibitors such as ABA. This effect of salinity could be partially alleviated by the application of exogenous growth-promoting substances (Khan et al., 2000). Intensive trials have been aimed at overcoming the drastic effect of salt stress on plants using various growth-promoting substances such as GA_3, IAA or kinetin (Singh et al., 1994; Khan and Unger, 2001). These authors reported that the interaction between salt stress and these substances exerted mostly positive effects, alleviating the adverse effects of salt stress on the growth of plants.

The aim of the present work was to evaluate the salt tolerance of three sorghum cultivars and their different responses to salinity stress with regard to leaf growth, RWC and TI of leaves, as well as the differential uptake of minerals (Ca^{2+}, K^+ and Na^+) and the K^+/Na^+ ratio in the different organs of sorghum cultivars. In addition, the interactive effect of salinity and shoot spraying with IAA was also investigated.

Materials and methods

The seeds of the sorghum (*Sorghum bicolor* L.) cultivars Dorado, Hagen Shandawil and Giza 113 were obtained from the Breeding Program of the Agricultural Research Center, Giza, Egypt. Weighed plastic pots measuring about 2400 cm^3 were filled with dried clay soil, and planted with 15 uniform seeds each in three replicates (pots) for each treatment. The pots were irrigated with water and left until the seedlings emerged. Thereafter, the pots were watered with NaCl solution to the salinization levels: 0 (control), 44, 88 and 176 mM NaCl. The plant shoots were sprayed twice with an aqueous solution (25 ppm) of IAA, 3 and 10 days after irrigating the seedlings with NaCl solution. The pots were left to grow in a growth chamber with a thermoperiod of 32/28°C day/night and a 12-h photoperiod. The growing plants were daily irrigated with water to maintain the desired salinization levels.

At the end of the experimental period (45 days), the plants were separated into roots, stems and leaves. The oldest and youngest leaves were also separated. Leaf area was determined by the disk method (Watson and Watson, 1953).

The tolerance index (TI) was calculated according to De Le Rosa-Ibarra and Maiti (1995):

$$TI = \frac{\text{Dry mass of plant under stress}}{\text{Dry mass of plant in the control}} \times 100$$

The relative water content was calculated according to Smart (1974) using the formula:

$$RWC\ \% = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

The fresh weight of the leaf samples was determined, after which they were floated on distilled water for up to 4 h to record the turgid weight. The dry weight of the leaves was determined after drying to constant mass in an aerated oven at 70°C. The Na^+, K^+ and Ca^{2+} contents of the roots, stems and leaves were determined with a flame photometer (Williams and Twine, 1960). The data of all experiments were subjected to analysis by the least significant differences test (L.S.D.) using the SPSS program.

Results

There were considerable differences in the leaf area, dry mass, relative water content (RWC) and tolerance index (TI) of the leaves between the three sorghum cultivars (Table 1) when subjected to salinity stress. Cultivars Dorado and Hagen Shandawil tolerated salinity up to 88 and 44 mM NaCl, respectively, but above this level, and at all salinity levels in Giza 113, a significant reduction in these parameters was observed. This reduction was more obvious in Hagen Shandawil and Giza 113 than in Dorado. The relative water content of Dorado was higher at all salinity levels than that of Hagen Shandawil or Giza 113. IAA treatment stimulated the leaf area, RWC, TI and dry mass of the leaves, as compared with the corresponding untreated plants.

Table 1

Effect of salinity and shoot spraying with IAA (25 ppm) on leaf area (LA) (cm^2 $plant^{-1}$), dry mass (DM) (g $plant^{-1}$), relative water content (RWC%) and tolerance index (TI) of 45-day-old plants of sorghum cultivars

Treatments	NaCl (mM)	Dorado				Hagen Shandawil				Giza 113			
		LA	DM	RWC%	TI	LA	DM	RWC%	TI	LA	DM	RWC%	TI
NaCl	0	27.9	0.646	81.1	1.00	27.5	0.618	78.7	1.00	25.1	0.623	72.1	1.00
	44	25.8	0.638	80.6	0.99	23.7	0.580	77.9	0.94	18.5**	0.516	70.3*	0.83*
	88	22.8	0.593	80.9	0.92	17.1**	0.445**	73.7**	0.72**	013.5**	0.383**	65.5**	0.61**
	176	14.0**	0.375**	72.0**	0.58**	10.0**	0.241**	67.6**	0.39**	9.8**	0.137**	60.4**	0.22**
NaCl	0	32.1	0.697	83.7**	1.08	33.2**	0.687	79.6	1.11	31.4**	0.656	75.7**	1.05
+ IAA	44	29.5	0.675	83.2**	1.04	28.1	0.625	78.5	1.01	24.4	0.538	74.9**	0.86
	88	25.0	0.648	82.8*	1.00	20.5**	0.581	75.9**	0.94	26.0	0.425*	71.6	0.68**
	176	17.4**	0.405**	82.4	0.63**	15.0**	0.383**	71.2**	0.62**	11.1**	0.218**	64.0**	0.35**
L.S.D.	5%	5.7	0.132	1.5	0.24	4.2	0.119	1.6	0.19	4.2	0.156	1.8	0.17
	1%	7.7	0.177	2.1	0.33	5.6	0.160	2.1	0.25	5.6	0.210	2.5	0.23

*Significant differences (P = 0.05) and **Highly significant differences (P = 0.01) as compared with the control (0 mM NaCl)

The differences in Na^+ and K^+ concentrations in the oldest and youngest leaves (Table 2) showed that the Na^+ contents were significantly increased, while the K^+ contents were significantly reduced in both the oldest and youngest leaves of the three sorghum cultivars under salinity stress, as compared with the control. The youngest leaf was able to maintain higher K^+ content than the oldest leaf in all three sorghum cultivars. Consequently, the K^+/Na^+ ratio was higher in the youngest than in the oldest leaf, and in Dorado than in Hagen Shandawil or Giza 113, especially at the highest salinity level. Shoot spraying with IAA retarded Na^+ absorption and promoted K^+ uptake. This effect of IAA was more obvious in Dorado than in the other two cultivars and in the youngest than the oldest leaf, as compared with the values for untreated cultivars.

Table 2

Effect of salinity and shoot spraying with IAA (25 ppm) on the Na^+ and K^+ contents (mg g^{-1} dry matter) and the K^+/Na^+ ratio of the oldest and youngest leaves of 45-day-old plants of sorghum cultivars

Treatments	NaCl (mM)	Dorado			Hagen Shandawil			Giza113		
		Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
Oldest leaf										
NaCl	0	5.49	25.92	4.72	7.32	11.14	1.52	10.37	16.20	1.56
	44	7.32*	30.50**	4.17	9.15*	9.54	1.04*	17.69**	10.08**	0.57**
	88	10.98**	30.06**	2.74	12.81**	9.14*	0.71**	23.18**	7.74**	0.33**
	176	17.08**	21.78**	1.28**	15.25**	7.74**	0.51**	31.96**	4.32**	0.14**
NaCl	0	6.71	28.44*	4.24	6.10	12.90*	2.11*	9.20	18.90**	2.05*
+ IAA	44	6.13	30.60**	4.99	7.32	10.62	1.45	13.42**	14.94	1.11**
	88	8.76**	35.28**	4.03	8.54	10.14	1.19	15.81**	16.92	1.07*
	176	10.08**	26.46	2.63*	12.81**	8.90**	0.69**	24.03**	9.11**	0.38**
L.S.D.	5%	1.67	1.92	1.56	1.77	1.62	0.47	1.75	1.63	0.41
	1%	2.26	2.61	2.12	2.40	2.20	0.63	2.37	2.21	0.55
Youngest leaf										
NaCl	0	6.71	59.76	8.91	7.32	25.30	3.46	10.93	20.22	1.85
	44	7.93	75.78**	9.56	8.54	22.56**	2.64	14.64**	18.54	1.27*
	88	11.20**	69.48**	6.20**	10.93**	18.54**	1.70**	17.69**	15.08**	0.85**
	176	22.57**	53.20**	2.36**	29.28**	19.98**	0.68**	29.28**	11.44**	0.39**
NaCl	0	4.27**	61.38	14.37**	6.71	29.08**	4.33	10.20	20.44	2.00
+ IAA	44	7.32	75.6**	10.33	7.64	28.42**	3.72	11.64	23.04**	1.98
	88	8.54*	70.16**	8.22	9.15*	25.28	2.76	13.42**	21.14**	1.58
	176	14.64**	61.92*	4.23**	15.13**	20.24**	1.34**	21.35**	12.24**	0.57**
L.S.D.	5%	1.64	1.73	1.55	1.67	1.69	1.09	1.45	1.87	0.55
	1%	2.23	2.35	2.10	2.26	2.30	1.47	2.02	2.54	0.75

* Significant differences (P = 0.05) and ** Highly significant differences (P = 0.01) as compared with the control (0 mM NaCl)

The contents of Na^+, K^+ and Ca^{2+} (Table 3) recorded in sorghum cultivars in response to salinity stress revealed that there was a marked increase in the concentration of Na^+, while the K^+ and Ca^{2+} contents were markedly decreased, except in the stem of Dorado. Salinity stress did not induce any changes in Ca^{2+} content in the leaves of the three sorghum cultivars as compared with the control. The K^+ content was higher in Dorado than in Hagen Shandawil or Giza 113, and in the stem and leaves than in the root. Consequently, the K^+/Na^+ ratio appeared to decrease under salinity stress (to less than 1) in the roots and to increase (to more than 1) in the stem and leaves (Table 4).

Table 3

Effect of salinity and shoot spraying with IAA (25 ppm) on the mineral composition (mg g^{-1} dry matter) in the root, stem and leaves of 45-day-old plants of three sorghum cultivars

Treatments	NaCl (mM)	Root			Stem			Leaves		
		Na^+	K^+	Ca^{++}	Na^+	K^+	Ca^{++}	Na^+	K^+	Ca^{++}
Dorado										
NaCl	0	14.61	23.76	10.33	18.87	64.26	12.56	10.35	65.34	11.66
	44	16.14	20.52**	8.91	21.31**	66.95	11.90	12.17*	70.11*	10.65
	88	19.18**	18.67**	7.15**	24.96**	66.20	12.00	17.66**	67.51	10.20
	176	28.31**	15.66**	7.96**	32.35**	64.17	17.21**	26.44**	56.87**	10.65
NaCl	0	13.39	26.10**	11.33	16.44**	66.60**	12.38	9.13	70.92	15.16**
+ IAA	44	13.70	21.33**	9.83	18.87	71.64**	12.91	10.35	61.83	14.35**
	88	14.31	17.19**	7.36**	17.35	66.44	12.35	14.91**	59.22**	11.76*
	176	17.96**	18.26**	8.23*	30.75**	65.77	20.66**	21.31**	62.28	13.66**
L.S.D.	5%	2.22	1.32	1.63	1.56	2.72	2.35	1.77	4.08	1.42
	1%	2.98	1.78	2.19	2.11	3.68	3.17	2.39	5.52	1.91
Hagen Shandawil										
NaCl	0	12.81	7.20	11.07	17.69	49.68	12.05	10.98	67.77	8.52
	44	12.51	8.37	9.70*	20.74**	44.91**	10.99	13.73**	62.73**	8.28
	88	24.05**	8.84	7.00**	22.54**	39.42**	10.93	21.05**	54.54**	9.62
	176	34.04**	9.09	6.33*	26.23**	33.30**	9.75**	25.67**	51.66**	9.72
NaCl	0	12.51	11.79**	11.47	16.47	32.22**	12.47	9.76	66.24*	8.63
+ IAA	44	12.81	10.44**	10.75	16.78	32.22**	12.17	12.81*	77.94**	9.37
	88	14.03	13.91**	7.55**	26.54**	33.03**	11.87	18.30**	56.43**	10.18*
	176	13.73	16.38**	8.02**	25.93**	39.55**	10.85	19.22**	69.75**	10.98**
L.S.D.	5%	1.91	1.94	1.16	1.46	1.05	1.62	1.49	1.19	1.37
	1%	2.57	2.16	1.57	1.97	1.41	2.18	2.01	1.60	1.84
Giza 113										
NaCl	0	13.42	9.36	11.11	24.40	22.86	9.81	14.64	45.93	11.45
	44	14.03	11.34**	7.85**	29.28**	22.14	9.59	16.16	37.98**	11.13
	88	22.26**	6.39**	7.75**	30.80**	21.66	9.38	29.28**	37.84**	11.20
	176	28.65**	5.85**	6.20**	30.80**	20.52**	7.18**	28.97**	33.57**	9.11**
NaCl	0	12.50	9.90	12.48	22.26	35.82**	10.48	11.59**	46.26	14.25**
+ IAA	44	11.59	7.47**	8.75**	28.67**	27.72**	10.70	16.16	43.83	12.48
	88	13.42	8.64	8.15**	28.67**	29.43**	10.66	17.69**	53.73*	11.58
	176	17.69**	7.92**	7.10**	18.60**	22.15	8.11*	23.48**	55.17**	10.11
L.S.D.	5%	2.82	1.02	1.63	2.68	1.31	1.38	1.94	2.97	1.37
	1%	3.80	1.37	2.20	3.61	1.76	1.86	2.61	4.02	1.84

* Significant differences (P = 0.05) and ** Highly significant differences (P = 0.01) as compared with the control (0 mM NaCl)

Table 4
Effect of salinity and shoot spraying with IAA (25 ppm) on the K^+/Na^+ ratio in the root, stem and leaves of 45-day-old plants of three sorghum cultivars

Treatments	NaCl (mM)	Dorado			Hagen Shandawil			Giza 113		
		Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
NaCl	0	1.63	3.41	6.31	0.56	2.81	6.17	0.70	0.94	3.14
	44	1.27**	3.14	5.76*	0.67	2.16**	4.57**	0.81**	0.76**	2.35*
	88	0.97**	2.65**	3.82**	0.38**	1.75**	2.59**	0.29**	0.70**	1.29**
	176	0.55**	1.98**	2.15**	0.27**	1.27**	2.01**	0.20**	0.67**	1.16**
NaCl	0	1.95**	4.05**	7.76**	0.94**	1.96**	6.79**	0.79*	1.61**	3.99*
+ IAA	44	1.56	3.80**	5.97	0.81**	1.92**	6.08	0.64	0.97	2.71
	88	1.20**	3.83**	3.97**	0.99**	1.24**	3.08**	0.64	1.03	3.04
	176	1.02**	2.14**	2.92**	1.19**	1.51**	3.63**	0.45**	1.19**	2.35*
L.S.D.	5%	0.19	0.29	0.47	0.14	0.18	0.42	0.08	0.11	0.67
	1%	0.25	0.39	0.63	0.19	0.24	0.56	0.10	0.15	0.91

* Significant differences (P = 0.05) and ** Highly significant differences (P = 0.01) as compared with the control (0 mM NaCl)

The total sodium content (root + stem + leaves) was markedly increased, while the total potassium content was markedly decreased in sorghum cultivars at increasing salinity levels as compared with the control (Table 5). In general, the sodium contents were higher and the potassium contents were lower in the salt-sensitive cultivar Giza 113 than in the more or less salt-tolerant cultivars (Dorado and Hagen Shandawil). It is interesting to observe that the ratio of the K^+ in the root and stem to the total K^+ was higher in Dorado than in Hagen Shandawil or Giza 113, while the opposite pattern of changes was recorded in the leaves. In addition, the K^+/total K^+ ratio was higher in the stem and leaves than in the root, while the Na^+/total Na^+ ratio was more or less unaffected in any of the three sorghum cultivars.

The application of IAA markedly retarded the accumulation of Na^+ and partially alleviated the inhibitory effect of salinity on the contents of K^+ and Ca^{2+} and on the K^+/Na^+ ratio in the different organs of sorghum cultivars as compared with the values for untreated plants.

Discussion

The different extents to which the leaf area was reduced in three sorghum cultivars subjected to salinity stress supported the conclusions of Munns et al. (1982) and Marcelis and Van Hooijdonk (1999), who suggested that the reduction in the leaf area during salt stress may be due to a reduction in leaf expansion, probably due to the effect of NaCl on cell division and/or cell expansion. Munns (1993) proposed that the death of old leaves due to the uptake

of salts in the tissue would inhibit the supply of nutrients or hormones to emerging leaves, and thus reduce leaf area. The salt tolerance of sorghum cultivars has been related to the capacity to increase succulence and RWC (Leidi and Saiz, 1997; Azooz, 2002). However, there were positive correlations between the RWC and both the dry mass and the tolerance index (TI) of the leaves, denoting that (i) Dorado had consistently higher RWC in the leaves than Hagen Shandawil or Giza 113 and (ii) Dorado and Hagen Shandawil tolerated salinity up to 88 and 44 mM NaCl, respectively, while Giza 113 showed no tolerance to salinity stress. Accordingly, the three tested sorghum cultivars responded to salinity stress as follows, in order from the most to the least tolerant, Dorado > Hagen Shandawil > Giza 113.

Table 5

Effect of salinity and treatment with IAA on the total Na^+ and K^+ (root + stem + leaves) (mg g^{-1} dry matter) and the ratios of Na^+/total Na^+ and K^+/total K^+ in the root, stem and leaves of 45-day-old plants of three sorghum cultivars

Treat-ments	NaCl (mM)	Total Na^+	Total K^+	Root		Stem		Leaves	
				Na^+/t. Na^+	K^+/t. K^+	Na^+/t. Na^+	K^+/t. K^+	Na^+/t. Na^+	K^+/t. K^+
Dorado									
NaCl	0	43.83	153.36	0.333	0.154	0.430	0.419	0.236	0.426
	44	49.62	157.58	0.325	0.130	0.429	0.425	0.245	0.444
	88	61.80	152.38	0.310	0.131	0.403	0.464	0.285	0.443
	176	87.10	136.70	0.325	0.114	0.371	0.469	0.303	0.416
NaCl	0	38.96	163.62	0.343	0.159	0.421	0.407	0.234	0.433
+ IAA	44	42.92	154.80	0.319	0.137	0.439	0.462	0.241	0.399
	88	46.57	142.85	0.307	0.120	0.372	0.465	0.320	0.414
	176	70.02	146.31	0.256	0.124	0.439	0.449	0.304	0.425
Hagen Shandawil									
NaCl	0	41.48	124.65	0.308	0.057	0.426	0.398	0.264	0.543
	44	46.98	116.01	0.266	0.072	0.441	0.387	0.292	0.540
	88	67.64	102.80	0.355	0.085	0.333	0.383	0.311	0.530
	176	85.94	94.05	0.396	0.096	0.305	0.354	0.298	0.549
NaCl	0	38.74	110.25	0.322	0.106	0.425	0.292	0.251	0.600
+ IAA	44	42.20	120.6	0.303	0.086	0.397	0.267	0.303	0.646
	88	58.87	103.37	0.238	0.134	0.450	0.319	0.310	0.545
	176	58.88	125.68	0.233	0.130	0.440	0.314	0.326	0.554
Giza 113									
NaCl	0	52.46	78.15	0.255	0.119	0.465	0.292	0.279	0.587
	44	59.47	71.46	0.235	0.158	0.492	0.309	0.271	0.531
	88	82.34	65.89	0.270	0.096	0.374	0.328	0.356	0.574
	176	88.42	59.94	0.324	0.097	0.348	0.342	0.327	0.560
NaCl	0	46.35	91.98	0.269	0.107	0.480	0.389	0.250	0.502
+ IAA	44	56.42	79.02	0.205	0.094	0.508	0.350	0.286	0.554
	88	59.78	91.80	0.224	0.088	0.479	0.320	0.295	0.585
	176	59.77	85.24	0.295	0.092	0.311	0.259	0.392	0.647

The application of IAA led to a significant increase in the values of these parameters, and the adverse effects of high levels of salinity were partially alleviated. This is in agreement with the findings of Khan et al. (2000). At high salinities, a significant reduction in growth occurs because of the plant's inability to adjust osmotically, while specific ion toxicities may cause a significant reduction in growth. The balance of growth regulators could be changed at high salinities and this effect could be partially alleviated by the application of exogenous growth-promoting substances, in the present case by IAA.

The higher K^+ content in the youngest leaf than in the oldest leaf in the three sorghum cultivars, especially in Dorado, suggests the presence of an efficient retranslocating system (Jeschke, 1984; Leidi and Saiz, 1997), and the stem seems to have a role in increasing the flow of K^+ to young leaves, while restricting the flow of Na^+.

The much higher content of K^+ in Dorado than in Hagen Shandawil and Giza 113 is an indication of salt tolerance and osmotic adjustment (Erdei et al., 1990). Erdei et al. (1996) reported that sorghum (more tolerant to stress) possessed the capability for maintaining higher K^+ levels than maize (less tolerant to stress) under salt stress. Another major difference in the strategy of salt tolerance in the three sorghum cultivars may be associated with the differences in Ca^{2+} content. Dorado not only maintained high Ca^{2+} content but also increased the amount of calcium in the stem compared with the control. On the other hand, Giza 113 had insufficient amounts of Ca^{2+}. The recorded increase in Ca^{2+} in the stem of Dorado might play a role in the salt tolerance of this cultivar (Shaddad, 1990).

The differences in survival and growth were mirrored by varietal differences in the absorption and consequently in the accumulation of K^+ rather than Na^+ by the root, stem and leaves of the three sorghum cultivars. The present results showed that salinity had a much more stimulatory effect on the flux of K^+ from the root to the stem and leaves in Dorado than in Hagen Shandawil and Giza 113, while there was no significant difference between the cultivars in the accumulation and partitioning of Na^+. This is an explanation for the fact that the K^+/total K^+ ratio was higher in the stem and leaves than in the root, while the Na^+/total Na^+ ratio was more or less unaffected in the tested cultivars. The opposing gradients of these ratios gave evidence of specific transfer processes within the different organs of sorghum cultivars.

Treatment with IAA induced, in most cases, a significant reduction in the absorption and accumulation of Na^+, but increased the K^+ and Ca^{2+} contents. This suggested that the exogenous application of IAA could play an important role in osmoregulation, increasing the efficiency of water utilization under stress conditions and thus increasing the salt tolerance of the experimental plants, while also maintaining the level of these ions in adequate amounts to enhance the metabolic processes of these plants.

Therefore, the present results are not in agreement with the results of many authors, who associated the differences in salt tolerance with the Na^+ criteria $^+$. Thus, Gorham and Young (1996) reported that salt-tolerant cultivars accumulated less Na^+ than sensitive cultivars, while Fortmeir and Schubert (1995) found the opposite pattern, where salt-tolerant cultivars accumulated more Na^+ than salt-sensitive ones.

It is of special interest in this work that the K^+ content in the whole plant of the three sorghum cultivars appeared to be proportional to the production of dry mass and the RWC of the leaves. This again confirmed that the salt tolerance of the three sorghum cultivars could be associated with the K^+ level rather than Na^+.

However, the use of the K^+/Na^+ ratio as an index of salt tolerance is still problematical, and could be different for different plants, different cultivars or even for the different organs of the same plants. This is not surprising because of the diverse mechanisms involved in the adaptation of different cultivars to salinity (Yeo, 1983).

In conformity with this observation, when these cultivars were sprayed with IAA, the K^+/Na^+ ratio changed markedly and the ameliorative effect of IAA might be associated with the reduction of Na^+ accumulation rather than with K^+ in the salt-sensitive cultivar (Giza 113), while in the more salt-tolerant Dorado, it seemed to be associated with the enhancement of the absorption and translocation of K^+.

Besides the problematical behaviour of the K^+/Na^+ ratio at the level of the whole plant, the partitioning of Na^+ and K^+ between leaves of different ages also appeared to be involved in salinity resistance. There was a substantial difference in the distribution of K^+ and Na^+ in the oldest and youngest leaves. The K^+/Na^+ ratio was much higher in the youngest leaves than in the oldest, and in Dorado than in Hagen Shandawil or Giza 113. Thus, the high K^+ content in the youngest leaves of Dorado might play an important role in osmoregulation, which in turn increased the driving force for water flow, and consequently increased the RWC and growth of leaves in Dorado compared with the other two cultivars.

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MICROBIOLOGICAL IMPACT OF ATRAZINE POLLUTION IN RIVER SEDIMENT AND SOIL

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Atrazine is a frequently detected pollutant in agricultural soils, groundwater and surface waters. Microbial degradation was confirmed in soils, and recently several atrazine-degrading bacteria have been isolated. Degradation in aquifers, however, is not well understood, and to date, atrazine degraders have not been isolated from water. In the present study, the impact of atrazine was assessed in agricultural soil and river sediment and the composition of the atrazine-degrading bacterial community in the soil and sediment was compared. Atrazine pollution increased the number and diversity of the endogenous atrazine degraders in both environments. Proteobacteria were predominant atrazine degraders in soils, whereas the community of atrazine-degrading bacteria in sediment consisted mostly of coryneforms.

Keywords: atrazine, biodegradation, soil, river sediment

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine; Fig. 1) is a commercial selective *s*-triazine herbicide. It has been intensely used over the past 50 years, mainly in maize production and conifer plantations. As a consequence of widespread use, atrazine is frequently detected in soils, groundwater and surface waters in agricultural areas (Boyd, 2000). Though the acute toxicity of atrazine is low, repeated applications are suspected of causing lung, heart, muscle and endocrine damage. Thus, the environmental fate and effects of atrazine demand attention. It is generally considered to be recalcitrant, the half-life ranging from 40 to 180 days in surface soils, though this is much longer (near persistent) under anoxic conditions in subsurface soil, groundwater and sediment (Radosevich et al., 1996; Papiernik and Spalding, 1998). In surface water degradation is attributed mainly to photolysis, but the dominance of microbial biodegradation was confirmed in soils (Kolpin and Kalkhoff, 1993; Ames and Hoyle, 1999).

Recently, several microorganisms capable of the biotransformation or mineralization of atrazine were isolated from agricultural soils with a long atrazine history (Yantze-Kontchou and Gschwind, 1994; Topp et al., 2000). The usual degradative pathway is dechlorination (formation of hydroxyatrazine), followed by the one- or two-step hydrolysis of amino alkyl groups to form cyanuric acid, and occasional ring cleavage (Fig. 1). The exact pathway and the

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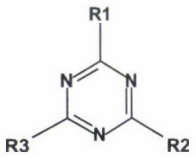
end metabolites are strain-specific. The atrazine degraders described so far include a wide range of bacteria (e.g. various species of the genera *Rhodococcus* and *Pseudomonas*, *Agrobacterium radiobacter*, *Clavibacter michiganensis*, *Pseudaminobacter* sp., *Nocardioides* sp.), and some white rot fungi (e.g. *Phanerochete* sp.).

Previous studies mainly focused on atrazine pollution in the soil, with the emphasis on the isolation of atrazine-degrading organisms and factors influencing the degradation of atrazine in the environment. The above-mentioned atrazine-degrading species were isolated from soil, either from agricultural sites with a long history of atrazine application or from atrazine spill areas. Little data is available on the environmental fate and impact of atrazine in aquatic and subhydric environments (Anderson et al., 2002).

In the present study, the effect of long-term atrazine pollution is compared in river sediment and soil. Atrazine degradation, its principal metabolites, and the composition of the atrazine-degrading bacterial communities were investigated and compared.



Compound	R1	R2	R3
Atrazine	-Cl	isopropylamine	ethylamine
Hydroxyatrazine	-OH	isopropylamine	ethylamine
Deethylatrazine	-Cl	isopropylamine	$-NH_2$
Deisopropylatrazine	-Cl	$-NH_2$	ethylamine
Ethylammelide	-OH	$-NH_2$	ethylamine
Isopropylammelide	-OH	isopropylamine	$-NH_2$
Ethylammeline	-OH	-OH	ethylamine
Isopropylammeline	-OH	isopropylamine	-OH
Ammeline	-OH	$-NH_2$	$-NH_2$
Ammelide	-OH	-OH	$-NH_2$
Cyanuric acid	-OH	-OH	-OH

Fig. 1. Chemical structure of atrazine and its main metabolites

Materials and methods

Sampling and sample collection

Soil samples were collected at a former herbicide storage and distribution site at Ják (Vas County, Hungary, N 47° 8', E 16° 35'). Careless handling and accidental spills polluted the area with a variety of pesticides, including atrazine, for over 30 years. Seven sampling sites were chosen randomly (T1–T7). At each site the surface debris was cleaned off, then composite samples from a depth of 10–40 cm were collected in sterile sampling bags. The samples were stored at 4°C till processing.

Atrazine degradation in water was studied in a laboratory-scale model system of the Danube river sediment bed. The model system (Fig. 2) is a simulation of bank-wall filtration containing native Danube sediment core samples, constructed for the investigation of microbial processes during filtration (Vargha et al., 2000). The model was operated under close to natural conditions. Danube water was fed in at 2.5–5 ml cm^{-2} day^{-1}. Six parallel sediment columns were investigated, of which one (S5) was fed with Danube water amended with 100 μg l^{-1} atrazine to simulate severe atrazine pollution. An unpolluted column (S2) was used as a control. Water samples were collected from the effluent water and side samplers (Fig. 2).

Sample chemical analysis

The soil samples were extracted by solvent extraction, using a 1:1 acetone/hexane eluent, and sonication. The water samples were extracted on solid phase extraction (SPE) columns with an octadecyl-silica phase. The extracts were analysed for atrazine and primary metabolites using reverse-phase high performance liquid chromatography-mass spectrometry (RP-HPLC/MS) as previously described (Takáts et al., 2001).

Microbiological evaluation

The total germ count and the germ count of atrazine degraders were determined by the 5-tube most probable number (MPN) method. Tenfold dilution series were prepared from the samples and inoculated into the appropriate liquid media: (i) minimal salt medium amended with atrazine as a sole source of carbon (AA broth: K_2HPO_4 0.8 g, KH_2PO_4 0.2 g, NaCl 0.2 g, $CaCl_2 \times 2$ H_2O 0.1 g, $MgSO_4 \times 7$ H_2O 0.2 g, $ZnSO_4 \times 7$ H_2O 0.03 g, $FeSO_4 \times 7$ H_2O 0.01 g, $CuSO_4 \times 5$ H_2O 0.02 g, $MnCl_2 \times 4$ H_2O 0.015 g, H_3BO_3 0.015 g, $Na_2MoO_4 \times 2$ H_2O 0.01 g, atrazine 0.1 g, $(NH_4)_2SO_4$ 0.6 g, distilled water 1 l, pH 7.2) , (ii) nutrient broth (peptone 5 g, meat extract 5 g, distilled water 1 l). In the nutrient broth, growth was detected turbidimetrically, while the AA broth was amended with 10 mg l^{-1} resasurine redox indicator to indicate growth, since undissolved atrazine (solubility is 30 mg l^{-1} at 25°C) interfered with the turbidity readings. In effluent water samples, the viable count of atrazine degraders was too low for the MPN result to be accepted as adequate, so plate counts on AA plates were simultaneously determined.

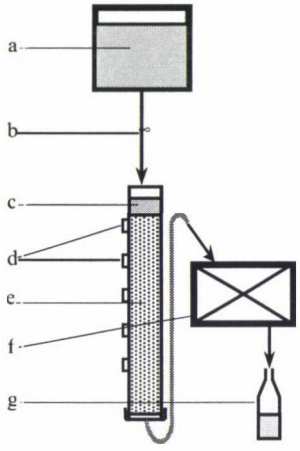


Fig. 2. Laboratory model system of Danube river sediment filtration
(a: water tank, b: adjustable inlet, c: water above the sediment, d: side samplers, e: sediment core, f: peristaltic pump, g: outlet sample collector)

Putative atrazine degraders were isolated on solid growth media containing atrazine as a sole source of nitrogen and/or carbon (AM or AA plates, respectively). The medium composition was similar to the AA broth described above but 0.6 g of atrazine was used, supplemented with 18 g agar for both media. This was amended with $(NH_4)_2SO_4$ 0.6 g l^{-1} for the AA agar and with 1 g l^{-1} mannose for the AM agar. The water samples were plated without dilution, while the soil samples were adequately diluted in distilled water. The plates were incubated at 28°C for 1 week. Colonies showing considerable growth after 1 week (>2 mm) were selected, and pure cultures were obtained, characterized and identified. Standard methods (Greenberg et al., 1992) were applied for the phenetic characterization of the strains: colony (size, colour, shape, consistency) and cell morphology (size, shape, presence and form of cell aggregates); Gram staining and endospore formation; and basic biochemical tests (oxidase and catalase reaction, Hugh-Leifson carbon source utilization test). The strains were identified by 16S ribosomal DNA sequence comparison (Stackebrandt and Goodfellow, 1991).

Atrazine degradation in liquid culture

Eleven sediment isolates showing rapid growth on atrazine were inoculated into AM liquid minimal media (described above) with atrazine as the sole source of nitrogen and the samples were processed after 2 weeks. The cells were broken by sonication and cell debris was removed by centrifugation. The supernatant was extracted by SPE and analysed by HPLC-MS as previously described (Takáts et al., 2001).

Results and discussion

Degradation of atrazine in soil

The soil type of the investigated area was brown forest soil. The texture, colour, organic matter content, etc., of the samples confirmed the inhomogeneity of the area. The colour ranged from yellowish brown to grey. The soil texture was loam or sandy loam, and total organic matter ranged from moderate to high. The ratio of carbon and nitrogen shifted from 12 to 8, presumably due to the pollution. The pH was slightly acidic (6.3–6.8). The atrazine concentration in the samples ranged between 0.46 to 86 μg kg^{-1} (Table 1). The first value is not significantly higher than the usual concentration detected in agricultural soils. The highest value, however, is almost 30 times higher than the EPA limit for soils (3 μg l^{-1}). Considering that no new pollution occurred for more than 10 years prior to sampling, the presence of such high residual amounts of atrazine suggests extremely high initial concentrations. Degradation did, however, occur, as confirmed by the presence of atrazine metabolites. The accumulated metabolites were found to be hydroxyatrazine and ethylammelide. Based on previous observations, the pH and the high concentration of other available nitrogen sources may account for the slow degradation of atrazine. Rousseaux et al. (2001) observed that at pH below 6.5 the biodegradative activity of the soils significantly decreased. The presence of easily utilized nitrogen forms was also shown to inhibit the degradation of atrazine (Abdelhafid et al., 2000). The hydrolysis of atrazine to hydroxyatrazine in soils was attributed earlier to an abiotic process (Harris, 1967); however, recent investigations confirmed the dominance of microbial activity (Ames and Hoyle, 1999). Degradation involving

dealkylation is uniformly accepted as the evidence of microbial degradation, so the presence of ethylammelide confirms biodegradation in the present samples. The latter metabolite is a characteristic degradation product of the most widespread bacterial metabolic pathway described initially in the *Pseudomonas* ADP strain (Boundy-Mills et al., 1997).

Concentration of atrazine and atrazine metabolites in the Danube

Investigations on the atrazine concentration in Danube water and bank-wall filtered well water were previously published by Takáts et al. (2001). Danube water contained only atrazine and hydroxyatrazine, while a wide range of dealkylated metabolites were detected in the well water (deethylatrazine, deisopropylatrazine, isopropylammelide and ethylammelide). The atrazine concentration decreased significantly, from 450 ng l^{-1} in Danube water to 50 ng l^{-1} in well water, though the total triazine concentration decreased only moderately (from 750 to 620 ng l^{-1}). According to our current knowledge, the abiotic transformation of atrazine (for instance by photolytic hydrolysis; see below) yields only hydroxyatrazine; thus, the presence of dealkylated derivatives indicates the presence of endogenous atrazine-degrading microbial activity in the Danube sediment (Giardi et al., 1985).

Atrazine and metabolites in the sediment model system

The 110 mm diameter sediment core samples consisted predominantly of sand and sandy gravel (44.7% gravel, 52.4% sand, 2.9% loam, <1% clay, organic matter content 1.22%, pH 7.8–8.0). The concentration of atrazine and atrazine metabolites in the effluent water was studied after 5 months of continuous simulated atrazine pollution. Water inflow was approximately 1 l day^{-1} containing 100 μg l^{-1} atrazine. The concentration decreased to approx. 10% of the initial value during filtration, and an average of 10(±4) μg l^{-1} was detected in the effluent water after 5 months of adaptation. During the following month this value remained relatively stable. Hydroxyatrazine was detected as a major metabolite at a concentration of 10(±3) μg l^{-1}, similar to that of the residual atrazine. Previous investigations in aquifers (surface water and water-saturated soil) also found hydroxyatrazine to be the principal product of degradation (Lerch et al., 1995; Mersie et al., 1998). Some authors attribute the formation of hydroxyatrazine to photolytic hydrolysis in surface water (Kolpin and Kalkhoff, 1993). Since the present model was operated in complete darkness, a photolytic reaction is highly unlikely, so hydroxyatrazine was presumably a microbial product.

The adsorption of atrazine on sediment particles may also influence its concentration. The accumulation of atrazine (up to 200 μg kg^{-1}) in soil and submerged soil due to binding to soil particles was reported by Németh-Konda et al. (2002) and Mersie et al. (1998). Previous investigations on the present

sediment model system indicated that the Danube sandy gravel sediment had limited atrazine-binding capacity (less than 2 μg kg^{-1}). The slight accumulation of hydroxyatrazine is possible, but adsorption cannot account for the absence of further metabolites.

The contradiction between observations *in situ* and in the model system can be resolved by two possible explanations: (i) atrazine-degrading microorganisms are distributed unevenly in the sediment, and those capable of dealkylating the compound are characteristic of the deeper regions; (ii) in the sediment column, the microbial community was adapted to high amounts of atrazine. Since monoalkyl metabolites are more readily degraded than hydroxyatrazine, it is possible that further metabolites were completely mineralized by the adapted bacteria and thus only the most stable, hydroxyatrazine, was detected. The first hypothesis is contradicted by the fact that, to our present knowledge, the biodegradation of atrazine is mainly aerobic, and generally a significant decrease in microbial degradation is observed with decreasing redox potential. Nor does it account for the loss of cumulative triazine in the model. The second hypothesis is supported by the finding that the isolated strains were all capable of both dealkylation and dechlorination of atrazine (see below).

Effect of atrazine on microbial counts

In the soil samples, the total counts were variable, ranging between 10^5 and 10^9 CFU g^{-1}, while the number of atrazine degraders ranged from 10^4 to 10^6 CFU g^{-1} (Table 1). The correlation between the total count values and the soil parameters was determined to evaluate the effect of atrazine pollution. The total count values exhibited a high correlation with the organic matter (especially the organic nitrogen) content of the soil samples ($r = 0.818$), but the correlation with the pollutant content was low (0.289). On the other hand, the number of atrazine degraders was correlated with the atrazine concentration (0.736), but not with the other soil parameters measured.

In bank-wall filtered well-water, the bacterial count is usually significantly lower than that of the river water, due to filtration through the gravel bed. The same effect was observed in the model system, where the total count decreased from 10^5 CFU ml^{-1} in the inflow Danube water to 10^2 CFU ml^{-1} in the effluent from all the sediment columns (Table 2). Atrazine pollution did not affect the total count. However, a distinct increase was observed in the number of atrazine degraders. The effect of atrazine on the "morphological diversity" of the plates was even more pronounced. One or two colony types were observed in the control samples, whereas up to 12 colony morphotypes were distinguished in the case of column S5.

Table 1
Atrazine and its metabolites in the soil samples investigated. Exact concentrations of atrazine metabolites were not determined because of the strong interference of other pollutants (e.g. gasoline)

Sample	Atrazine concentration	Presence of metabolites		Total count	Atrazine degraders
	$\mu g\ kg^{-1}$	Hydroxyatrazine	Ethylammelide	CFU g^{-1}	CFU g^{-1}
T1	0.78	+	–	2.5×10^5	2.5×10^4
T2	12.40	+	+	1.7×10^7	3.5×10^4
T3	1.18	+	–	2.0×10^6	3.0×10^4
T4	86.20	+	+	2.0×10^6	1.7×10^6
T5	2.07	+	–	3.0×10^5	9.5×10^4
T6	41.50	+	+	5.0×10^9	3.0×10^6
T7	0.46	+	–	3.5×10^5	3.5×10^4

Table 2
Total count and count of atrazine-degrading bacteria from the effluent of the sediment columns S5 was adapted to atrazine for 5 months prior to the examination

Columns	Total count, CFU ml^{-1}	Atrazine degraders, CFU ml^{-1}
S1	3.5×10^2	1.4×10^2
S2	1.3×10^2	7.0×10^1
S3	2.5×10^2	2.3×10^2
S4	7.0×10^1	2.7×10^1
S5	3.5×10^2	1.1×10^3
S6	1.2×10^2	1.6×10^2
Inflow	3.0×10^5	4.5×10^2

Comparison of atrazine-degrading communities in soil and water

Twenty-five strains were isolated from soil samples on media with atrazine as the sole source of nitrogen or carbon. Restriction analysis on the 16S rDNA revealed 15 different groups. Though the isolates exhibited varying colony morphology (e.g. colour ranging from white through yellow and orange to dark brown), the cell morphology and basic biochemical reactions were uniform. All the strains were Gram-negative, non-spore-forming, motile rods, and were oxidase- and catalase-positive.

The sediment isolates were more diverse, with dominant features differing from those observed in the soil isolates. Thirteen of the 17 maintainable strains were Gram-positive, pleomorphic organisms, with varying morphological and biochemical characteristics.

The identification of the isolates further emphasized the difference between the atrazine-degrading communities in sediment and soil. The majority of the soil bacteria belonged to the Proteobacteria division, except for an unidentified isolate (AAD4) from the *Cytophaga-Flexibacter* group. The genus *Pseudomonas* was most numerous (16 out of 25, belonging to 8 rDNA groups) and four were various *Stenotrophomonas* strains (2 rDNA groups), while the

remainder were representatives of the genera *Achromobacter*, *Rhizobium*, *Sphingomonas* and *Variovorax* (Fig. 3). The sediment isolates were mainly coryneform organisms (*Rhodococcus* [4 rDNA groups], *Corynebacterium*, *Microbacterium*, *Aeromicrobium*), and a few other Gram-positives (*Bacillus*, *Deinococcus*, *Micrococcus*). The Gram-negative strains were *Delftia*, *Pseudomonas*, *Alcaligenes* and *Xanthomonas* species (Fig. 4).

Such a pronounced difference was not expected, especially as a previous study had demonstrated that pseudomonads were predominant in the sediment column (prior to atrazine pollution) as well (Vargha et al., 2000). However, at both sites, representatives of several previously described atrazine-degrading genera were observed, such as *Pseudomonas* (de Souza et al., 1995), *Rhizobium* (previously *Agrobacterium*) (Struthers et al., 1998), *Rhodococcus* (Shao and Behki, 1995), *Alcaligenes* and *Stenotrophomonas* (Rousseaux et al., 2001). Further isolates belonged to genera that were associated with the degradation of other pesticides, e.g. diclofop-methyl (Smith-Geeier and Adkins, 1996), pentachlor-phenol (Lee et al., 1998), 2,4-D (Vedler et al., 2000) and propachlor (Villareal et al., 1991).

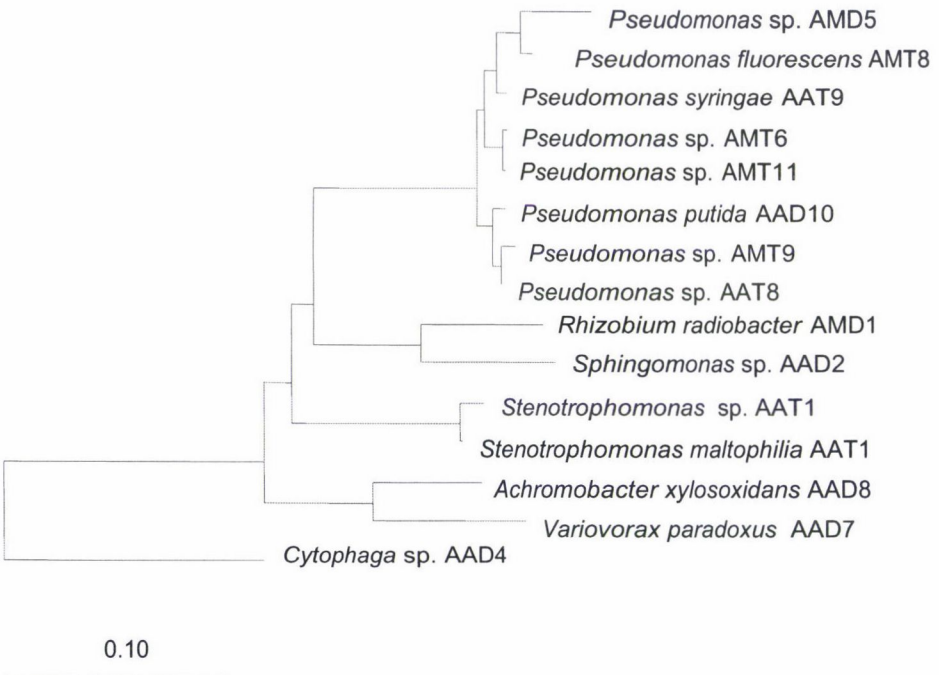


Fig. 3. Phylogenetic tree of the atrazine-degrading soil isolates.
One isolate per rRNA group is shown. Bar represents 10 base substitutions per 100 base pairs

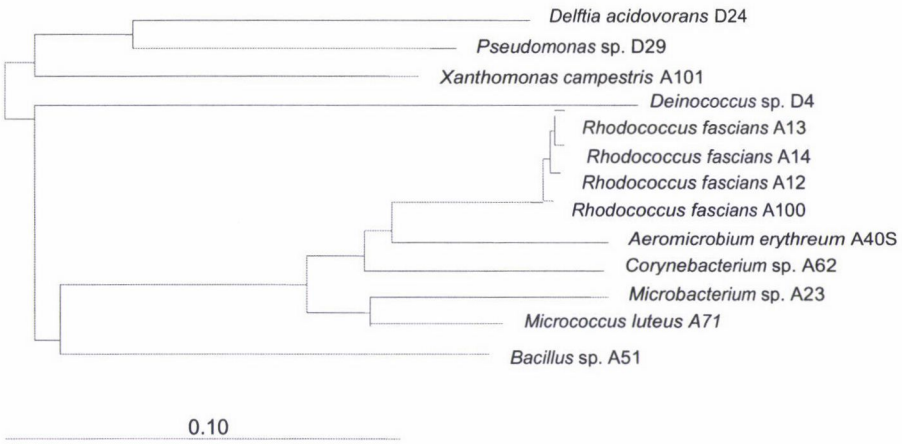


Fig. 4. Phylogenetic tree of the atrazine-degrading sediment isolates (unidentified isolates are not shown). Bar represents 10 base substitutions per 100 basepairs

Atrazine metabolism of sediment isolates

Since all the previously described strains were isolated from a soil environment, the metabolism study focused on sediment isolates (Table 3). All the investigated strains were able to transform atrazine to hydroxyatrazine. Ten out of the eleven strains also hydrolysed least one of the N-alkyl substituents, yielding monoalkyl-ammeline or -ammelide as a terminal product. The simultaneous removal of the aminoalkyl residue, a distinguishing step in the well-characterized and widespread atrazine degradation pathway of *Pseudomonas* sp. ADP, was not observed (Boundy-Mills et al., 1997). Biuret, assumed to indicate cleavage of the triazine ring, was only produced by *Delftia acidovorans* D24 (Table 3).

Table 3
Atrazine metabolites detected in liquid culture of the Danube sediment isolates

Strains	Detected atrazine metabolites								
	1	2	3	4	5	6	7	8	9
Rhodococcus sp. A12	–	–	+	+	+	+	–	–	–
Rhodococcus sp. A13	+	–	+	+	+	+	+	–	–
A13P unidentified	–	–	+	+	+	–	+	+	–
Rhodococcus sp. A14	–	–	+	+	+	+	+	–	–
Microbacterium sp. A23	–	–	+	–	–	+	+	–	–
Aeromicrobium sp. A40	+	–	+	+	+	+	+	–	–
Bacillus sp. A51	–	–	+	–	–	–	–	–	–
Micrococcus luteus A 71	–	–	+	+	–	+	+	–	–
A140 unidentified	–	–	+	–	+	+	+	–	–
Deinococcus sp. D4	+	+	+	+	+	–	–	–	–
Delftia acidovorans D24	–	–	+	+	+	+	+	+	+

1: Deisopropylatrazine; 2: Deethylatrazine; 3: Hydroxyatrazine; 4: Ethylammeline; 5: Isopropyl-ammeline; 6: Ammeline; 7: Ammelide; 8: Cyanuric acid; 9: Biuret

Conclusions

Atrazine degradation was studied in the soil of a former herbicide storage site and in an artificially polluted laboratory model system of the Danubian gravel bed. Biodegradation by endogenous microbial communities was observed in both environments, hydroxyatrazine and ethylammelide (only in soil) being the principal metabolites accumulated. Atrazine pollution did not affect the total viable bacterial count directly, but it had a pronounced effect on the composition of the microbial communities. The proportion of atrazine degraders increased. Strains isolated on atrazine as the sole source of nitrogen or carbon usually belonged to species or genera previously associated with pesticide degradation. The soil isolates were predominantly Proteobacteria, while coryneform organisms were most prominent in the sediment. Sediment isolates were found to degrade atrazine via diverse metabolic pathways, differing in part from those previously observed in soil bacteria.

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EFFECT OF SOILBORNE WHEAT MOSAIC VIRUS ON WINTER WHEAT YIELD AND YIELD COMPONENTS

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Soil-borne wheat mosaic virus (SBWMV) is an important disease of wheat production areas throughout the world, causing a great reduction in wheat and barley yields. The most effective way of controlling the disease is the use of resistant varieties in infested areas. In this study, the effects of SBWMV on yields and some yield components of eight susceptible, one moderately susceptible/resistant and nine resistant varieties were evaluated using data from 9 virus-infested and 6 non-infested sites in Eskisehir, Turkey over 6 years. The susceptible varieties yielded 5.35% more than resistant varieties in non-infested sites, while they gave 28.98% lower yield in infested sites. Significant yield loss differences were observed between the varieties in infested sites. Decreases were also observed in yield components at various levels. This study showed that SBWMV is an important disease, which survives in soil for long periods and causes significant yield decreases in wheat. Recently developed varieties have good resistance to the disease and are recommended to farmers in infested areas.

Key words: wheat, soil-borne wheat mosaic virus, yield loss, resistance
Abbreviations: SBWMV, soil-borne wheat mosaic virus

Introduction

The introduction of several high-yielding spring and winter wheat varieties in the early 1970s caused the occurrence of wheat diseases which had not been seen before in wheat production areas in Turkey. The two most important of these diseases were *Septoria tritici* on spring wheat and SBWMV on winter wheat. SBWMV was first seen on a Bezostaya 1 field in the village of Fevziye in the Alpu Valley, Eskisehir, Turkey in 1971 (Altay and Karma, 1973). Club wheat varieties, such as Ak702, had been grown in this region previously and after the introduction of Bezostaya 1, the disease was observed on nearly 20,000 ha. Disease surveys showed that, in addition to the Alpu Valley, infested areas were also found in other areas of Eskisehir and Konya (Bolat, 1994).

According to Koehler et al. (1952) and McKinney (1953), SBWMV was first reported by Illinois researchers in Illinois, USA in 1919. Later it was observed in most of the soft and hard red wheat growing regions in the USA, Japan, Italy, Egypt and Brazil (Dickson, 1956; Palmer et al., 1974). McKinney (1953, 1967) identified yellow mottling, light green and rosette forms of the disease symptoms. Pakumbaba et al. (1971) studied the physical properties of the yellow (common) strain. Although both forms are affected by the same environmental conditions (Webb, 1927), the mottling form has less impact on the yield than the rosette form (Campbell et al., 1975).

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Depending on the year, field and variety grown, disease symptoms can be observed as shapeless patches of different sizes. These patches include dark green colour on the leaves, dwarfing, stunting, excess tillering, and thickening at the base of the stem. Plants sometimes have yellow-green mosaic leaves in early spring. Later in the growing season infected plants have the appearance of non-vernalized spring-sown winter wheat plants. Some of them may start heading but they have delayed maturity. Similar symptoms have been reported from wheat growing areas in the USA (Dickson, 1956; McKinney, 1967; Myers et al., 1993). Up to 100% yield loss may be observed depending on the severity of infection. Several studies have been conducted to determine the effect of SBWMV on the yield and yield components of wheat (Campbell et al., 1975; Hunger and Sherwood, 1985; Hunger et al., 1989; Kucharek and Walker, 1974; Nykaza et al., 1979; Palmer and Brakke, 1975). The yield loss in winter wheat due to the disease is affected by virus strains, varieties grown, management techniques and the environment (Brakke et al., 1987; Nykaza et al., 1979; Larsen et al., 1985; Webb, 1927).

SBWMV is transported through the resting spores or zoospores of an obligate parasitic fungus *Polymyxa graminis* Led. It can survive inside the fungus in infected plant debris for more than 11 years (Brakke, 1971). The fungus itself does not have any adverse effect on infected plants (Rao, 1968). The virus is rod-shape, 20 nm in diameter and consists of long and short particles (Brakke, 1971). These particles are 110–160 nm and 300 nm long, and the length of the short particles depends on the virus strain (Palmer et al., 1974). It requires both particles to cause the disease (Tsuchizaki et al., 1975). In addition to wheat, SBWMV can infect rye, barley and some species of *Bromus* (Palmer et al., 1974; Rao and Brakke, 1968).

P. graminis infects wheat roots when the weather is cool and moist in autumn. The length of this cool period in autumn and in early spring affects the severity of the disease (Nykaza et al., 1979). If the temperature is below 17°C for a long period, the severity of the symptoms increases. The recovery of the plants is faster when the temperature is higher than 17°C. Web (1927) reported that the disease occurs in the range of 10 to 16°C. Sowing time, soil temperature, amount of inoculum and plant genotype also have an effect on disease development (Brakke and Estes, 1967; Campbell et al., 1975; Hunger and Sherwood, 1985; Hunger et al., 1989). Roots are infected simultaneously with SBWMV and the transporting fungus, after which the virus starts to move into the plant. The virus and the fungus can be disseminated by wind, water and farm equipment.

Although it is known that resistance is controlled by a single dominant gene, the repair, regrowth and recovery of the plants are affected by the environment and the genetic background of the varieties (Rashied et al., 1982). SBWMV was isolated from the roots of both resistant and susceptible varieties when they were grown in infested soil (Myers et al., 1993). This shows that this is not a case of resistance to the vector; the fungus enters the roots of both resistant and susceptible plants. However, the virus can only be detected from the leaves of susceptible plants in later development stages. When the virus was

inoculated on to leaves of susceptible and resistant plants, it was detected only in plant parts below the inoculation point in resistant plants, but both below and above this point in susceptible plants (Ramjuan and Lapierre, 1992; Myers et al., 1993). Resistance can thus be explained by the fact that although the vector infects the roots of both susceptible and resistant plants, virus movement to upper parts is restricted in resistant ones.

SBWMV can be avoided by late planting, but this strategy also causes an economically significant yield reduction in wheat. The use of resistant varieties in virus-infested areas is the most cost-effective way of controlling the disease (Armitage et al., 1990). Varieties resistant against SBWMV have been developed and are available for use in these areas (Makkouk et al., 1994; Rashied et al., 1982; Myers et al., 1993; Nykaza et al., 1979; Dickson, 1956; Hunger and Sherwood, 1985; Bolat, 1994).

The objective of this study was to evaluate yield losses by and varietal responses to the disease in the long term.

Materials and methods

A total of eighteen wheat varieties with different responses to SBWMV were used in the experiments (Table 1). Each year 10 or 12 varieties, half of which were susceptible and the other half resistant, were used and newly developed varieties were added to the experiments when available, while some old ones were discarded after at least two growing seasons. A total of 15 experiments were carried out in 7 locations with a history of SBWMV infestation and on 6 non-infested test sites in six growing seasons between 1978–79 and 1997–98 (Table 2). The selected infested and non-infested sites were adjacent or very close fields. A randomized complete block design with four replications was used at all sites. Planting was done in the first weeks of October. Planting density was 500 seed/m^2 and plot sizes at harvest were 6 m^2. Seed treatment for smut and soil insects, fertilizer and herbicide applications were done according to recommended farm applications. A susceptible check, Bezostaya 1, was planted round the borders of all the plots to observe pathogen variations in the soil. The plots were harvested in the first weeks of July.

The varieties were classified into R (resistant) and S (susceptible) groups according to whether they could reach the booting stage. Only one variety, Ak702, was accepted as MS-MR since it showed both reaction types in some years. Recovery grouping was determined according to the numbers of spikes with normal appearance at the flowering stage.

The evaluation of the yield and yield components of varieties grown under virus-infested and non-infested conditions was performed using the formula:

$$Lx = (Xn–Xi) – (\overline{Rn} – \overline{Ri})$$ (Campbell et al., 1975)

Where:

Lx = Yield loss of a susceptible (X) variety due to SBWMV

Xn = Yield of variety X on the non-infested site

Xi = Yield of variety X on the infested site

Rn = Average yield of resistant varieties on the non-infested site

Ri = Average yield of resistant varieties on the infested site

This formula considers resistant varieties as indicators and eliminates environmental factors other than disease (Campbell et al., 1975; Miller et al., 1992). Percentage losses for the yield and yield components of the varieties were calculated using the formula:

$$\% \text{ loss} = 100Lx / (Xi + Lx)$$

Yield losses were calculated for each location and are presented in Table 2.

The JUMP statistical program was used to analyse the data.

Table 1
Names, SBWMV reactions, recovery in early spring, and maturity of the wheat varieties used in the experiments

Variety	Origin	Reaction*	Recovery	Maturity
Bolal 2973	Turkey (Cultivar)	S	Poor	Mid-early
Kırac 66	Turkey (Cultivar)	S	Very poor	Late
Gerek 79	Turkey (Cultivar)	S	Poor	Mid-early
Bezostaya 1	Russia (Cultivar)	S	Very poor	Mid-early
Yektay 406	Turkey (Cultivar)	S	Fair	Mid-early
4-11	Turkey Cultivar)	S	Very poor	Mid-early
Kutluk 94	Turkey (Cultivar)	S	Poor	Mid-late
Kirgiz 95	Turkey (Cultivar)	S	Poor	Mid-early
Ak 702	Turkey (Land Race)	MS-MR	Very good	Late
Zincirli	Turkey (Land Race)	R	Good	Late
Domanic	Turkey (Land Race)	R	Good	Late
ES14 (Hys/7C)	Turkey (Advanced Line)	R	Good	Mid-early
Dibo/Mfo	USA (Advanced Line)	R	Good	Mid-early
Edch/Lfn	USA (Advanced Line)	R	Good	Mid-early
Haymana 79	Turkey (Land Race)	R	Good	Mid-early
ES 86-7	Turkey (Cultivar)	R	Good	Mid-late
Suzen 97	Turkey (Cultivar)	R	Good	Mid-early
Sultan 95	Turkey (Cultivar)	R	Good	Mid-late

*R: Resistant; MS-MR: Moderately susceptible-Moderately resistant; S: Susceptible

Table 2
Yield performances (t/ha) of susceptible and resistant varieties at contaminated and non-contaminated sites

Year	Location	SBWMV*	Average yield			Loss (%)	Significance+
			R	S	R/S (%)		
1979	Fevziye	Infested	3.14	2.52	80.3	19.7	**
	Fevziye	Non-infested	4.98	5.50	110.4		
1980	Alpu	Infested	3.86	1.73	44.8	55.2	**
	Alpu	Non-infested	2.50	2.60	104.0		
1981	Karacay	Infested	3.52	3.10	88.1	11.9	**
	Karacay	Non-infested	3.21	3.43	106.9		
1993	Aktepe	Infested	1.92	1.55	80.8	19.2	
	Esenbel	Infested	1.80	1.87	103.9		
	Hamidiye	Non-infested	2.02	2.07	102.5		** (1)
1994	Aktepe	Infested	1.88	1.69	89.9	10.1	
	Esenbel	Infested	1.46	1.67	114.4		
	Hamidiye	Non-infested	2.04	2.31	113.2		* (1)
1998	Yesildon	Infested	2.59	1.55	59.8	40.2	**
	Fevziye	Infested	1.93	0.00	0.0	100.0	**
	Hamidiye	Non-infested	3.19	3.01	94.4		

*infested or non-infested; R: resistant varieties; S: susceptible varieties; +Significance of susceptible vs. resistant varieties; (1) The mean yield of the susceptible group was higher than that of the resistant group at this non-infested test site

Results

Data on yield losses due to SBWMV, collected for 18 wheat varieties from 15 sites in six years, are summarized in Table 2. Averaged over six years, susceptible varieties yielded 5.35% more than resistant varieties on non-infested sites, while they yielded 28.98% lower on infested sites (Table 2). Yield losses changed significantly depending on year and location; the greatest yield loss observed was 100% in Fevziye in 1998, while the lowest yield loss due to the disease was 10.1% in Aktepe in 1994. However, susceptible varieties yielded 3.9 and 14.4% more than the resistant varieties in Esenbel, one of the SBWMV infested sites, in 1993 and 1994, respectively (Table 2). This was due to the tolerance of susceptible varieties to the Zn deficiency observed at this site, combined with a relatively dry period in these years. In addition, there was very little rainfall in the autumn of this year and it came too late for the plants to germinate (Table 3). This caused delayed germination and lower infection than in the other years. The data are presented in the light of this information. Low temperature and high precipitation in early spring play an important role in SBWMV disease development. These conditions were highly conducive to disease development in Fevziye in 1979, Yesildon in 1998 and Alpu in 1980. The highest yield losses were observed in these locations and years. In contrast, the temperature was 11.7°C and the precipitation amounted to 15.7 mm in 1994. This resulted in lower infection and the fast recovery of infected plants in the spring, leading to lower yield losses. Since varieties susceptible to SBWMV have higher yield potential than resistant ones in disease-free environments, higher yields were obtained from these varieties at non-infested sites, except in Hamidiye in 1998 (Table 2).

The yield losses incurred by individual varieties due to SBWMV are summarized for different test sites and years in Table 4. Except for the 100% yield loss for all susceptible varieties in Fevziye in 1998, Bezostaya 1 showed the highest yield loss of 81.6% in Alpu in 1980. Kutluk 94 followed with 61.5% yield loss in Yesildon in 1998. The highest average yield losses were obtained for Kutluk 94 and Kirgiz 95. However, these two varieties were tested at only two sites in 1998. Among the varieties most widely grown on the Central Anatolian plateau, Gerek 79, Bezostaya 1, Bolal 2973 and Kirac 66 had 33.04, 31.95, 28.29 and 26.72% average yield losses, respectively. The MS-MR variety, Ak702 exhibited lower yield loss.

The results of the limited studies on yield components in these experiments are presented in Table 5. The effect of SBWMV on test weights was variable in the three locations where yield component data were collected. A slight increase in test weight was observed in some susceptible varieties when compared to the resistant ones, depending on the test site, while others showed a slight decrease. This could be due to the more efficient use of late rainfall received in the spring by such susceptible varieties and the fast recovery of

infected plants due to that precipitation as an advantage of late maturation. The yield loss observed in each susceptible variety was the result of specific yield components that were more affected by SBWMV than the other components for that particular variety. For example, the seed number/floret, with a 21.5% decrease, was the principle component responsible for yield loss in Bolal 2973, while the florets/spike, with a 20.9% decrease, was the most important component in Kirac 66 (Table 5). For Gerek 79, Bezostaya 1, Yektay 406 and 4-11, the seed number/floret was the yield component most affected by SBWMV. The situation was different for Ak702, where the spike number/m^2 was the component most affected. This could be due to its heterogeneous structure and MR-MS reaction to SBWMV.

Table 3
Meteorological data in the months critical for SBWMV infection

Month	Meteorological data	1978–79	1979–80	1980–81	1992–93	1993–94	1997–98
October	Average temperature (°C)	12.2	12.6	12.9	14.5	12.8	12.2
	Soil temp. (5 cm depth)	12.4	13.0	12.4	14.4	14.9	14.7
	Precipitation (mm)	55.4	38.7	11.6	42.3	2.1	47.6
November	Average temperature (°C)	2.8	6.9	7.6	4.5	3.7	6.8
	Soil temp. (5 cm depth)	3.0	6.8	7.1	4.5	4.8	8.5
	Precipitation (mm)	0	38.6	33.1	29.8	56.1	17.3
April	Average temperature (°C)	9.4	9.2	9.6	9.8	11.7	12.8
	Soil temp. (5 cm depth)	11.5	10.8	12.0	10.9	13.6	15.0
	Precipitation (mm)	11.7	27.1	21.7	15.7	25.2	83.0
May	Average temperature (°C)	14.9	15.3	12.9	14.6	15.4	15.1
	Soil temp. (5 cm depth)	17.8	17.1	15.4	15.9	17.8	18.6
	Precipitation (mm)	61.4	31.3	53.2	50.6	35.8	152.7

Table 4
Yield losses observed in susceptible wheat varieties in different years and locations

Variety	1979	1980	1981	1993	1993	1994	1994	1998	1998	Average
	Fevziye	Alpu	Karacay	Aktepe	Esenbel	Aktepe	Esenbel	Fevziye	Yesildon	
Bolal 2973	41.5	55.0	8.3	3.99	0.04	26.8	11.3	100	19.9	28.29
Kirac 66	26.9	60.0	24.9	0.61	6.75	27.4	42.5	–	–	26.72
Gerek 79	–	53.2	20.5	0.60	6.22	32.9	23.9	100	27.0	33.04
Bezostaya 1	42.5	81.6	26.4	2.37	5.34	6.4	19.7	100	5.2	31.95
Yektay 406	26.2	39.8	5.6	–	–	–	–	–	–	23.03
4–11	19.60	49.2	14.2	–	–	–	–	–	–	26.80
Kutluk 94	–	–	–	–	–	–	–	100	61.5	80.75
Kirgiz 95	–	–	–	–	–	–	–	100	38.7	69.35
Ak 702	–	–	–	0.69	6.62	5.7	8.3	1.1	1.6	4.00

Table 5
Percentage increase (–) or decrease in the yield components of susceptible wheat varieties

Variety	Spikes per m^2	Florets per spike	Seeds per floret	TKW	Test weight		
	1993	1980	1980	1980	1980	1993	1993
	Aktepe	Alpu	Alpu	Alpu	Alpu	Aktepe	Esenbel
Bolal 2973	6.95	15.8	21.5	0.63	–10.05	1.40	–2.82
Kirac 66	15.20	20.9	0.8	10.08	–2.66	0.50	0.27
Gerek 79	16.49	–0.8	23.0	12.63	–2.25	–0.27	–0.69
Bezostaya 1	29.60	20.0	29.0	16.80	–2.51	3.56	1.34
Yektay 406	5.15	0.0	29.9	5.15	–4.10	–	–
4-11	8.54	4.6	19.0	8.54	–3.69	–	–
Ak 702	30.65	–	–	–	–	0.84	1.35

Discussion

The effects of SBWMV on the yield and yield components of wheat show significant differences depending on varieties and locations. This study was conducted at 15 sites in seven locations since the disease was first reported in the Alpu Valley, Eskisehir. Except for the test weights, the results obtained from this study were similar to the results of other studies reported on SBWMV (Campbell et al., 1975; Kucharek and Walker, 1974; Palmer and Brakke 1975). The disease caused decreases in the yield and yield components of wheat. Hunger et al. (1989) reported 27, 15, 12 and 8% decreases in the yield, tillering, test weight and plant height of susceptible varieties, respectively, due to SBWMV. They also observed a 10-day delay in maturity. Similar results were reported by Nykaza et al. (1979).

The reasons for the contrasting results on test weight in this study are not clear since the data were very limited. They could be due to the varieties used in the study, to management techniques or to some other reason.

Wheat is a main crop in the wheat-fallow system and there is no better alternative on the Anatolian plateau. Among the varieties used in this study, Bolal 2973, Kirac 66, Gerek 79 and Bezostaya 1 have a 20–30-year production history in the region and they are still the most widely-grown varieties. This is because of their high yield capacities and yield stabilities in this dry farming region. SBWMV is already causing a significant decrease in production in the area examined in the study. If it is not controlled, the problem will continue, with serious consequences.

Other methods suggested to control the disease (Kucharek et al., 1974; Pakumbaba et al., 1971) are not as effective as the use of resistant varieties in the infested areas. Resistant varieties have not been widely grown so far because of their lower yield performance compared to traditionally grown varieties. It is important to include SBWMV resistance studies in wheat breeding programmes for these regions.

The resistant varieties recently developed by the Anatolian Agricultural Research Institute, are recommended for the infested areas. Among them, Sultan 95, Suzen 97 and a recently developed variety, Altay 2000, have given good performances both in the infested and non-infested areas of the region.

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Short communication

TRENDS IN THE HEADING DATES OF WINTER EMMER [*Triticum turgidum* ssp. *dicoccon* (Schrank)] LANDRACES OF DIFFERENT ORIGIN IN THE GRADIENT GROWTH CHAMBER

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In the course of gene bank research, problems frequently arise when valuable genetic materials have to be multiplied in an environment where the climatic conditions are quite different from those in its original habitat. In recently commenced experiments on the raising of emmer, the heading dates of two genotypes originating from different sources (MvGB 301 and MvGB 304) were investigated in a gradient growth chamber in the Martonvásár phytotron. This chamber allows precise information on the optimum temperature and light requirements of plants in different developmental stages to be obtained during the growth of a single generation. The data indicated that MvGB 301 headed considerably later than MvGB 304 at all temperature levels, but both varieties headed normally even at a constant very low temperature of 8°C. It was found that the light intensity had no influence on the heading dates of the two varieties.

Key words: gradient chamber, heading date, *Triticum turgidum* ssp. *dicoccon*

Introduction

One of the problems frequently encountered in gene bank research is the multiplication and characterisation of valuable genetic materials originating from widely differing environments. Research begun in recent years on emmer (*Triticum turgidum* ssp. *dicoccon*) has been hindered on several occasions by the fact that the valuable accessions obtained from foreign gene banks could not be successfully multiplied either in the field or under artificial conditions. The climatic conditions in Hungary were alien to these populations and the temperature and/or drought stress experienced in the field in various stages of growth, combined with pathogens which were new to them, weakened the plants to such an extent that a large proportion of them died. In the majority of the remaining plants, heading was protracted even when they were sown in autumn. Similar problems were encountered in the phytotron, where many populations started to head and flower extremely late. In addition to genetic determination, the plant growth conditions also played an important role in this protracted heading and flowering. If the heading of emmer genotypes is to be genetically analysed, it is essential to provide reproducible plant growth conditions that will maximise the differences between the heading types represented by the various

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genotypes. These conditions can best be defined in gradient (inhomogeneous) systems. When the plants are grown in such a system it is possible to pinpoint the temperature and light conditions required for heading within a single generation and to determine what set of environmental conditions is best suited for demonstrating differences in heading date between two emmer genotypes.

In the present experiments, changes in the heading dates of two emmer populations with different growth types were studied using a low temperature gradient.

Materials and methods

In the present experiments two winter emmer [*Triticum turgidum* ssp. *dicoccon* (Schrank) Thell.] landraces of different origin (gene bank accessions MvGB 301 and MvGB 304) were examined. MvGB 301 originated from the eastern slopes of the Ukrainian Carpathian Mountains and MvGB 304 from the western slopes of the Caucasus Mountains. The basic experimental stock was developed using the previously reported technique (Kőszegi and Kovács, 2003) and randomly chosen husked grains were soaked, in their husks, at 20°C tap water for 12 hours. After vernalisation at 2°C for four weeks the seedlings were planted into pots, according to the methods generally used for artificial plant growth in the phytotron (Tischner et al., 1997). Pots containing the two varieties were then arranged in the gradient chamber of the Martonvásár phytotron (Tischner and Veisz, 1996) in alternate rows. The temperature in the rows was adjusted to give a gradient between 8 and 18°C, and the light intensity in the columns to a gradient between 210 and 540 μmol m^{-2} s^{-1} (Fig.1), with a daylength of 16 h (Kőszegi et al., 2003).

The dates of tillering, heading and flowering were scored for each plant throughout the experiment. Only the inner 10×10 matrix was evaluated. The results were analysed using multivariable statistical methods (SPSS 6.0, 1993). The present paper discusses the results obtained for heading.

Results and discussion

The heading dates of the two varieties, averaged for each temperature level, are presented in Table 1. The data clearly revealed a significant difference in the heading dates of the two genotypes at all the temperature levels.

Table 1

Changes in the heading dates of the two emmer genotypes as a function of the temperature gradient (number of days from planting)

Temperature °C*	MvGB 301		MvGB 304	
	Heading date	SD (mean)	Heading date	SD (mean)
8	110.7	2.6	88.0	2.6
↓	106.5	2.6	83.2	5.6
↓	101.8	2.0	78.4	9.4
↓	96.3	1.8	65.2	4.3
↓	84.0	0.8	57.6	2.3
↓	82.5	0.6	55.2	2.4
↓	77.5	5.2	52.4	3.1
↓	71.0	0.8	48.8	5.0
↓	70.2	6.4	44.2	0.4
18	66.2	2.6	44.0	0.8

* continuous temperature gradient, temperature difference between rows is approx. 1 °C

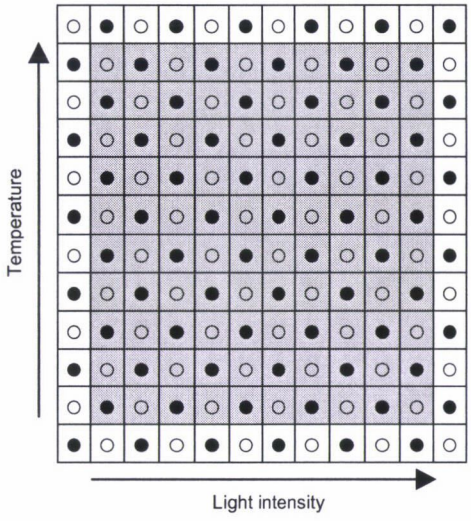

Fig. 1. Experimental design. ●: MvGB 301; ○: MvGB304

The data show that MvGB 301 headed considerably later than MvGB 304 at all the temperature levels. It was surprising to note, however, that both varieties headed normally even at a constant temperature of 8°C. It was also at this extremely low temperature that the greatest differences were observed between the two genotypes (Fig. 2).

This indicates that if the genes responsible for heading are to be mapped in crossing combinations of these two varieties, the tests should ideally be carried out at low temperature, since it is here that the difference between the two genotypes is most significant. Surprisingly, the light intensity had no influence on the heading dates of the two varieties (Fig. 3).

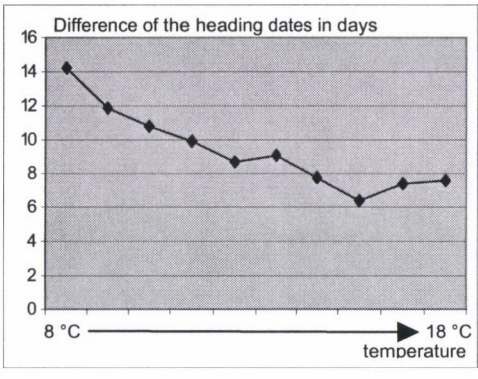

Fig. 2. Differences in the heading dates of the two varieties as a function of temperature

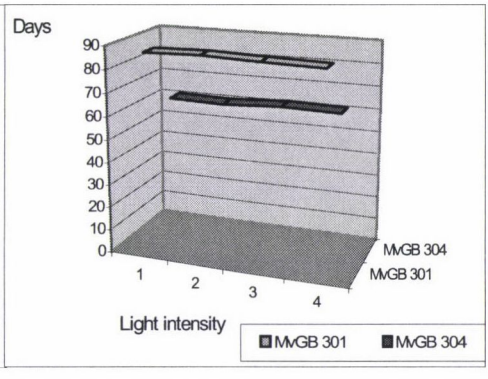

Fig. 3. Effect of light intensity on the heading dates of the two varieties

The fact that the intensity of illumination caused no change at all in the heading date indicates that the plants can be raised to heading at relatively low cost. The results clearly suggest that a combination of low temperature and low-intensity illumination is the most suitable for investigations on the heading date of emmer genotypes and on the inheritance of this trait.

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CHANGES IN THE ELECTROPHORETIC SPECTRA OF ANTIOXIDANT ENZYMES IN NITRATE-FED AND NITROGEN-FIXING SOYBEAN SUBJECTED TO GRADUAL WATER STRESS

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The effect of two sources of nitrogen (nitrogen fixation or nitrate assimilation) and gradual water stress on the electrophoretic spectra of peroxidase, catalase and superoxide dismutase was studied in soybean leaves. An increase in H_2O_2 production was observed, especially after the prolonged drought treatment. At 50% drought the activity of anionic peroxidase activity for isoenzymes Nos. 2 and 7+8 significantly increased (by 54 and 18%, respectively) in the leaves of nitrate-fed plants compared to the control plants; for nitrogen-fixing plants these values were 31 and 14%, respectively. In the case of cationic peroxidases, the application of 50% drought led to the inhibition of the moderately fast isoenzymes (Nos. 2 and 3, with Rm 0.5 and 0.65, respectively) and the activation of the fastest moving isoenzyme (No. 4, with Rm 0.8) in nitrate-fed soybean. The same tendency was observed in the leaves of nitrogen-fixing plants. The effect of restricted soil humidity on SOD activity was expressed as a change in the activity of some of the isoenzymes. There was a clear tendency for the SOD isoenzyme activity to increase after the exposure of nitrate-fed and nitrogen-fixing soybean plants to 50% drought treatment. High catalase activity was registered in control nitrate-fed plants. Generally the catalase isoenzyme activity in control nitrogen-fixing plants had low values. Both intensities of water stress (30 and 50% drought) caused an increase in the catalase activity, and this increase was much higher for nitrogen-fixing plants. Therefore, soybean plants responded to drought treatment by changes in the antioxidant enzyme activity, as these changes were partially dependent on the source of nitrogen. The results suggested that nitrogen-fixing soybean plants were more resistant to gradual water stress.

Key words: antioxidant enzymes, *Glycine max* L., hydrogen peroxide, nitrogen sources, water stress

Abbreviations: SOD – superoxide dismutase; ROS – reactive oxygen species; PAG – polyacrylamide gel

Introduction

Environmental stresses exert their effects either directly or indirectly through the formation of reactive oxygen species, ROS (Yu and Rengel, 1999). Along with various signalling molecules that modulate the stress response, ROS are involved in plant defence responses (Bolwell, 1999). For this reason antioxidant enzymes such as peroxidase, catalase and superoxide dismutase (SOD) have been used as biochemical markers for various types of biotic and abiotic stresses (Quiroga et al., 2001). An increase in enzyme activities under

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moderate drought stress could be indicative of increased ROS production and of the building up of a protective mechanism to reduce oxidative damage in plants subjected to water deficit (Yu and Rengel, 1999). The antioxidant enzymes also control the steady-state levels of moderately reactive oxygen species, allowing them to play an important role at specific sites, environmental conditions or plant developmental stages.

The main sources of ROS in plants under physiological conditions are respiration, photosynthesis and N_2 fixation (Matamoros et al., 2003). ROS are abundant during nodule formation and senescence. Root cells respond to rhizobial infection with an enhanced production of superoxide and H_2O_2 (Santos et al., 2001; D´Haeze et al., 2002; Ramu et al., 2002). SODs and catalases are critical for the protection of nitrogen fixation and occur in both symbiotic partners.

There have been many reports about the responses of nitrogen-fixing and nitrate-fed plants to limited water supply. Some authors (Djekoun and Planchon, 1991; Wery et al., 1994; Serraj et al., 1999) reported that nitrogen-fixing plants are more sensitive to drought stress than nitrate-fed ones. However, several studies (Antolin et al., 1995; Frechilla et al., 2000; Lodeiro et al., 2000) showed that the nitrogen-fixing species alfalfa, pea and common bean are more tolerant to water stress.

To answer the question of whether the nitrogen source affects the plant's resistance to drought treatment in some way, the activity of peroxidase, catalase and superoxide dismutase isoenzymes was determined in the leaves of soybean plants subjected to gradual water stress.

Materials and methods

Plant material and growth conditions

Soybean seeds (*Glycine max* L. Merr. cv Hodgson) were surface sterilized with 70% ethanol. The plants were cultivated in plastic pots containing 4 kg soil in a naturally illuminated greenhouse with a photoperiod of 15 h, and day/night temperatures of about 28–30°C/22–24°C. The plants were divided into two groups: 1. Nitrate-fed soybean plants grown in soil, where nitrate was maintained at a constant level of 12 mg NO_3^-/100 g soil at the 1st–8th trifoliate expanded leaves stage. 2. Nitrogen-fixing soybean plants (inoculated with a bacterial suspension of *Bradyrhizobium japonicum* strain 273 at approximately 10^8 viable cells per cm^3) grown in nitrogen-deficient soil, supplied with 4 mg NO_3^-/100 g soil until the 5th trifoliate expanded leaf stage.

Water stress application

Water stress was applied for 21 days during the 5th–8th trifoliate expanded leaf stage by decreasing the amount of watering. The plants were divided into four treatment groups: 1. Nitrate-fed control plants; 2. Nitrate-fed plants with a limited water supply; 3. Nitrogen-fixing control plants; 4. Nitrogen-fixing plants with a limited water supply. The plants received the equivalent of 80% of the transpirational water loss measured on day 0 from day 1 to day 7 of treatment (5th–6th trifoliate expanded leaf), 70% from day 8 to day 14 (6th–7th trifoliate expanded leaf) and 50% from day 15 to day 21 (7th–8th trifoliate expanded leaf). The plants were watered several times a day. Transpiration was determined by weighing the pots (Minguez and Sau, 1989). The surface of each

pot was covered with transparent plastic, so the loss of water by direct evaporation from the soil was negligible. All investigated parameters were recorded on days 0 (0% drought), 7 (20% drought), 14 (30% drought) and 21 (50% drought).

Hydrogen peroxide determination

The endogenous hydrogen peroxide was measured spectrophotometrically (λ=360 nm) after reaction with 1 M KJ according to Jessup et al. (1994). The results were calculated using a standard curve prepared with fresh hydrogen peroxide solutions.

Enzyme extraction and enzyme assays

The enzyme patterns were investigated on soybean leaf tissues. All steps in the extraction were performed at 4°C. The leaf material was homogenized with 0.1 M Tris-HCl buffer, pH 8.0 (1:3 w/v). Then the extract was centrifuged at 12 000 g for 30 min and the supernatant was used as a crude enzyme extract. The protein content was determined after trichloroacetic acid precipitation by the method of Lowry et al. (1951) with bovine serum albumin as a standard. The enzymes were separated by native electrophoresis in 7.5% polyacrylamide gel (PAG) according to Davis (1964). Enzyme protein spectra were scanned densitometrically (ERI-10, Germany). The differences and similarities of the individual protein bands were estimated by their number and by their relative electrophoretic mobility. Relative mobility (Rm) = a/b, where a is the distance (in cm) of the protein band from the start of the gel to its place on the gel; b is the distance (in cm) from the start of the gel to the front (marker dye – bromphenol blue). Equal amounts of enzyme proteins (100 µg) were run on the gels. The chemicals were purchased from Sigma Chemical Co. (St. Louis, USA).

Peroxidase activity

Peroxidase activity was detected by incubating the gels for 15 min in a reaction mixture consisting of 5 mM benzidine hydrochloride and 10 mM H_2O_2 in 0.05 M acetate buffer, pH 4.9 (Ornstein, 1964). The peroxidase isoenzymes were separated as described by Reisfeld et al. (1962).

SOD isoenzymes

SOD isoenzymes were stained on the gels by the method of Greneche et al. (1991). The gels were incubated for 30 min in the dark in a mixture consisting of 10 mg NBT, 75 mg EDTA-Na_2 and 3 mg riboflavin, dissolved in 100 ml Tris-HCl buffer, pH 8.2. They were then illuminated for 15 min, washed with distilled water and fixed with a 2:1:1:1 ratio of H_2O:ethanol:acetic acid:glycerol.

Catalase isoenzymes

Catalase isoenzymes were detected by the method of Woodbury et al. (1971). The gels were incubated in the dark for 20 min in 0.1 M K/Na phosphate buffer, pH 7.0 with 10 mM H_2O_2. The next step was incubation in a mixture of equal volumes of 2% $K_3Fe(CN)_6$ and 2% $FeCl_3$ for 15 min.

The area occupied by the peak of each isoenzyme was quantified and used as the basis for calculating differences in the isoenzyme activities of peroxidase, SOD and catalase.

Results

The study revealed that there is no significant difference in H_2O_2 content between nitrate-fed and nitrogen-fixing control plants (Fig. 1). With both nitrogen sources, drought treatment increased the H_2O_2 accumulation in plant leaves. On the last sampling day (50% drought), the H_2O_2 content of nitrate-fed plants was 41% higher compared to the control nitrate-fed plants. For stressed nitrogen-fixing plants this value was 40% higher than the control.

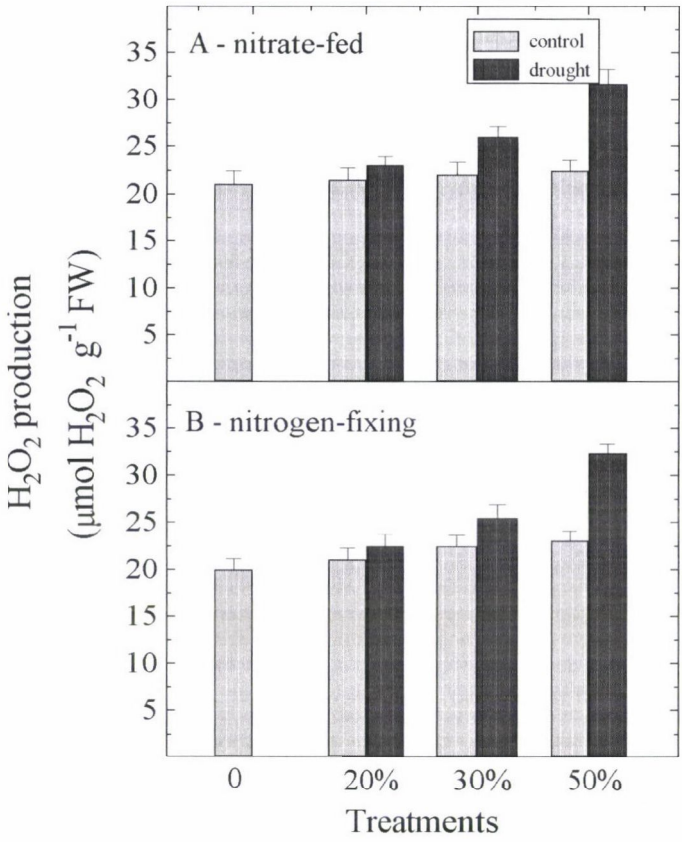

Fig. 1. Hydrogen peroxide production of nitrate-fed (A) and nitrogen-fixing (B) soybean plants. All investigated parameters were recorded on days 0 (0% drought), 7 (20% drought), 14 (30% drought) and 21 (50% drought). Values represent means ±SD (n=4)

The peroxidase in the leaves of all treatments was fractioned into 6–8 anionic isoenzymes (Fig. 2a-j). The most active were isoenzymes Nos. 7 and 8, which had the highest relative mobility (Rm 0.7). At the 7th trifoliate expanded leaf stage a significant enhancement (48, 95 and 65%, respectively) in the activity of the anionic peroxidase isoenzymes Nos. 2 (Rm 0.1), 4 (Rm 0.4) 7 and 8 was observed compared to the 5th trifoliate expanded leaf stage in nitrate-fed control plants (Fig. 2a, b), whereas at the 8th trifoliate expanded leaf stage the peroxidase activity of these isoenzymes decreased (by 36, 25 and 14%, respectively) (Fig. 2a, c). The isoenzyme spectra of nitrogen-fixing control plants (Fig. 2f–h) were very similar to those of nitrate-fed control plants. In the case of 30% drought the peroxidase activity of isoenzymes Nos. 2, 4 and 7+8 decreased (by 27, 51 and 27%, respectively) in nitrate-fed plants compared to the control plants (Fig. 2b, d). However, this parameter increased in nitrogen-fixing plants (Fig. 2g, i). In the 50% drought treatment the anionic peroxidase activity of isoenzymes Nos. 2 and 7+ increased 8 in nitrate-fed plants (by 54 and

18%, respectively) compared to the control plants (Fig. 2e, c), while in nitrogen-fixing plants this rise was 31 and 14%, respectively (Fig. 2j, h). At 30% drought, isoenzymes Nos. 2 and 4 were more active in nitrogen-fixing plants (by 41 and 209%, respectively) than in nitrate-fed ones (Fig. 2i, d). In the 50% drought treatment the activity of isoenzymes Nos. 2, 4 and 7+8 increased by 14, 80 and 30%, respectively, compared to the nitrate-fed plants (Fig. 2j, e).

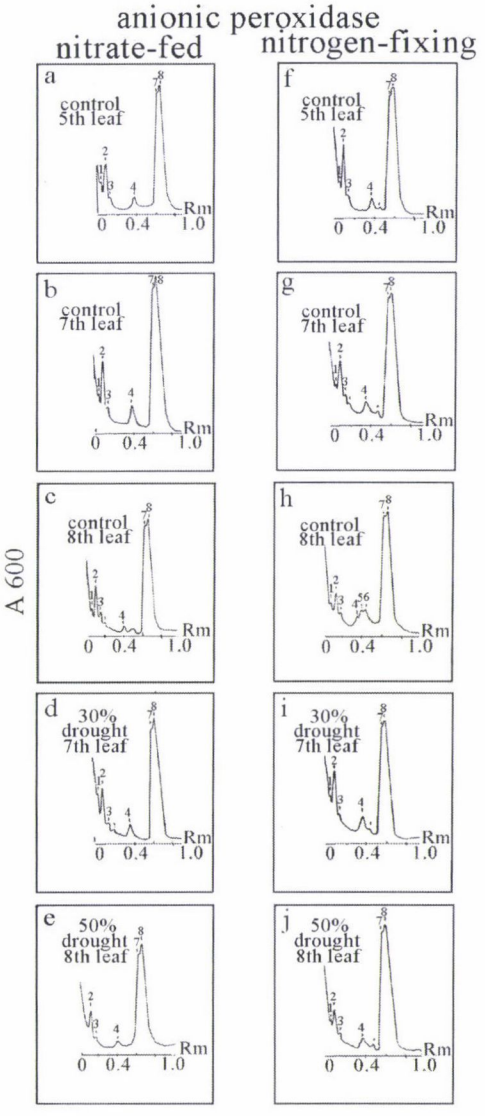

Fig. 2. Densitometric scans of anionic peroxidase isoenzymes from soybean leaves

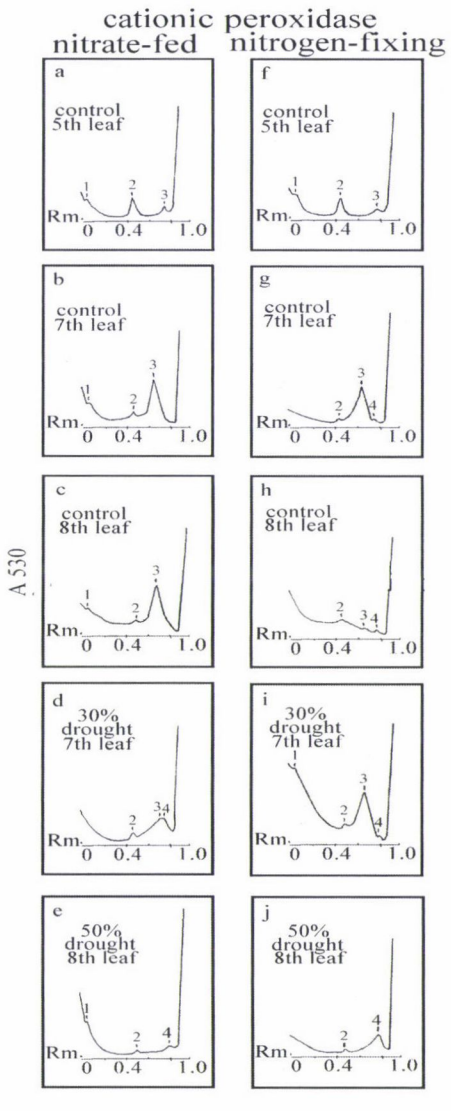

Fig. 3. Densitometric scans of cationic peroxidase isoenzymes from soybean leaves

There were 3–4 isoenzymes that characterized cationic peroxidase activity in soybean leaves (Fig. 3a–j). The activity of isoenzyme No 2 (Rm 0.5) declined during the development of control plants, but that of isoenzyme No. 3 (Rm 0.65) increased (Fig.3a–c, f–g). The only exception was izoenzyme No. 3 in nitrogen-fixing plants, which exhibited a decrease in activity at the 8th trifoliate expanded leaf stage (Fig. 3h). The application of 50% drought led to the inhibition of the moderately fast-moving isoenzymes (Nos. 2 and 3) and the activation of the fastest moving isoenzyme (No. 4; Rm 0.8) in nitrate-fed soybean (Fig. 3c, e). The same tendency was observed for nitrogen-fixing plants (Fig. 3h, j).

Eight well-distinguished SOD isoenzymes were revealed in the leaves of nitrate-fed plants (Fig. 4a-j). The bulk of the SOD activitity was distributed between the moderately fast-moving (Nos. 3, 4 and 5; Rm 0.6) and fast-moving (Nos. 6, 7, 8; Rm 0.7, 0.8 and 0.9, respectively) isoenzymes. The effect of restricted soil humidity on SOD activity was expressed as a change in the activity of some isoenzymes in the leaves during plant development (Fig. 4a-j). There was a clear tendency for the SOD isoenzyme activity to increase after exposure to 50% drought in nitrate-fed (Fig. 4c, e) and nitrogen-fixing (Fig. 4h, j) soybean plants. At 30 and 50% drought the percentage increase in SOD isoenzyme activity was much higher in nitrogen-fixing plants than in nitrate-fed soybean.

High catalase activity was registered in control nitrate-fed plants (Fig. 5a-c). Generally, the catalase isoenzyme activity in control nitrogen-fixing plants remained at a low level throughout the experimental period (Fig. 5f-h). Four slowly migrating isoenzymes were stained on the gels. The most active were those with Rm 0.4. The activity of the catalase isoenzymes changed during plant development and depended on the severity of the water stress. Both levels of water stress caused an increase in the catalase activity, but the increase was much higher for nitrogen-fixing plants (Fig. 5i, j). It should be noted that increases of 135 and 234% were observed for isoenzyme No. 1 and increases of 128 and 94% for isoenzymes Nos. 2+3+4 in nitrogen-fixing plants subjected to 30 and 50% drought compared to the respective controls.

Discussion

It is known that hydrogen peroxide can inactivate Calvin cycle enzymes and metalloproteins such as superoxide dismutase and nodule leghemoglobin (Scandalios et al., 1997). On the other hand, besides their direct detrimental effect on plants, ROS (H_2O_2, hydroxyl radical and superoxide radical) may have some benefit for the plant metabolism (Neil et al., 2002). The present results showed that under drought treatment, an increase in H_2O_2 production was observed, especially after exposure to prolonged drought treatment (last sampling day). Similar results were reported by Srivalli et al. (2003).

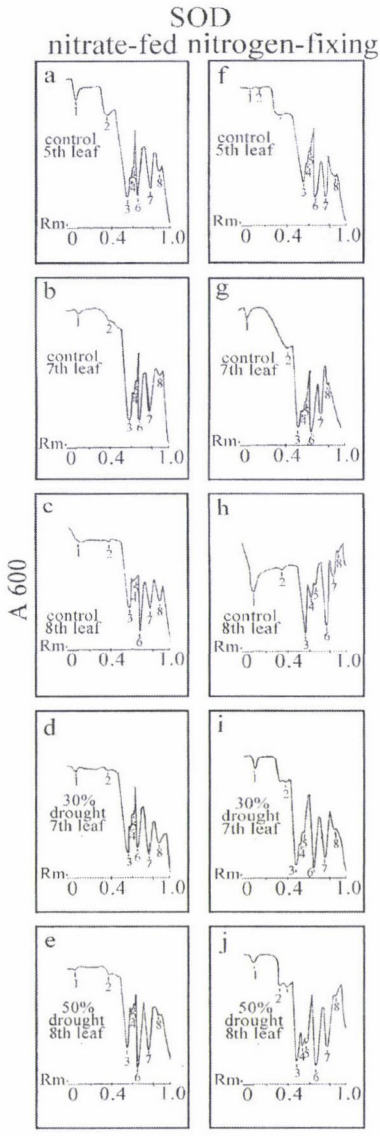

Fig. 4. Densitometric scans of SOD from soybean leaves

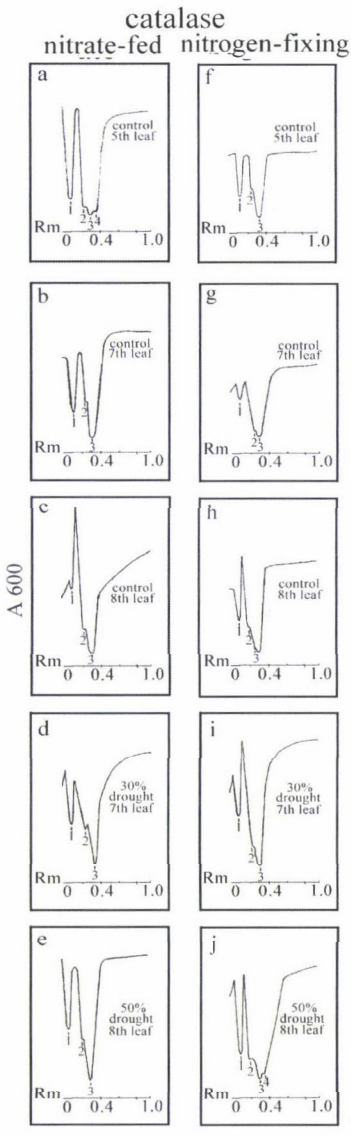

Fig. 5. Densitometric scans of catalase from soybean leaves

It is believed that under stress conditions, alterations in the cytoplasmic enzyme activity are often associated with the induction of plant antioxidant systems. Bacon et al. (1997) proposed that the increased activity of cell wall peroxidases may have an important role in controlling the cell expansion rate in plants grown with restricted water supplies. In the present study with cytosol anionic peroxidases, a significant increase in their activity was also found as a

response to two levels of drought. The activity of the isoenzymes of cationic peroxidases (IAA-oxidases) was also activated by 30% drought, but at 50% water deprivation their activity was greatly decreased.

Contradictory data on SOD responses to water deficit have been reported, ranging from decreased (Quartacci et al., 1994), through unaffected (Badiani et al., 1990) to increased levels (Luna et al., 1985) of enzyme activity. A change was observed in the present experiments in the activity of individual SOD isoenzymes in nitrate-fed and nitrogen-fixing plants during development under restricted soil humidity. In nitrogen-fixing plants an enhancement of SOD activity was observed in the prolonged drought treatment (50% water stress). Yu and Rengel (1999) and Quiroga et al. (2001) reported that the total SOD activity in lupin markedly increased with an increase in the level of drought stress. They observed a defence response of SOD isoenzymes to drought. When drought stress was relieved, the activity of CuZnSOD recovered to the control level, while the FeSOD activity remained above the control level. In contrast, the MnSOD activity was not affected by drought stress. Contrary to this study, Iturbe-Ormaetxe et al. (2001) reported that severe water stress caused a decrease in the activity of all the antioxidant enzymes in pea plants.

Different levels of catalase activity were found in nitrate-fed and nitrogen-fixing plants (Fig. 5a–j). In general, the catalase isoenzyme activity in leaf tissues of nitrogen-fixing soybean plants was low. High catalase activity was observed in the nodules of control soybean plants (Troitskaya et al., 2000; Santos et al., 2001; D'Haeze et al., 2002; Ramu et al., 2002). Catalase activity was investigated in nitrate-fed rice, wheat and cucumber seedlings exposed to oxidative stress and the results suggested that the fall in catalase activity was a phenomenon occurring in many plant species under these conditions (Ie-Sung et al., 2003). It was established in the present work that a more severe reduction in soil humidity (50% drought) caused a greater enhancement of isoenzyme activity in nitrogen-fixing plants than in nitrate-fed ones.

In conclusion, peroxidase, catalase and superoxide dismutase can efficiently detoxify ROS. Soybean leaves underwent changes in the antioxidant enzyme activities under drought treatment, and these changes were highly dependent on the stage of plant development. Exposure of plants to the higher level of water stress (50% drought) caused an increase in peroxidase, catalase and superoxide dismutase activity and this enhancement was more pronounced for isoenzymes in the leaves of nitrogen-fixing plants. On comparing nitrogen-fixing plants to nitrate-fed ones, it was concluded that nitrogen-fixing soybean showed higher resistance to gradual water stress, which may be considered as resistance to mild and moderate stress (Kirova et al. 2004).

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INTERACTIVE EFFECTS OF LIGHT, TEMPERATURE AND CULTIVAR ON PHOTOSYNTHESIS IN EVENING PRIMROSE (*OENOTHERA* SPP.) CROPS

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The photosynthetic performance of evening primrose (*Oenothera* spp.), a temperate oilseed crop, was assessed during the period of rapid biomass accumulation and flower bud formation. Light response curves constructed from field-grown plants harvested in late May, late June and late July were similar, suggesting that the photosynthetic capacity of evening primrose leaves is not readily susceptible to low temperatures. The maximum quantum efficiency of CO_2 assimilation and light-saturated rate of CO_2 assimilation data were comparable to other C_3 species. Short-term changes in photosynthetic efficiency, measured as the ratio of variable to maximal chlorophyll fluorescence, Fv/Fm, were assessed on field-grown plants of five breeding lines during late May and early June, and on glasshouse-grown plants under controlled temperatures and light levels. Low temperature-dependent photoinhibition (measured as a decline in Fv/Fm) occurred in both field and controlled-environment studies. Differences were observed between breeding lines in the rate of recovery upon a return to more favourable conditions. A clear correlation between Fv/Fm and CO_2 assimilation was demonstrated, suggesting that low temperature-dependent photoinhibition could lead to reduced biomass accumulation in evening primrose crops grown in cool temperate climates.

Key words: Chlorophyll fluorescence, CO_2 assimilation, evening primrose, light response curves, low temperature-dependent photoinhibition, *Oenothera* spp., photosynthesis, quantum efficiency

Introduction

Evening primrose (*Oenothera* spp.) is now established as a high-value oilseed crop which can be successfully grown in temperate areas of northern and central Europe, North America and Australasia (Simpson and Fieldsend, 1993). The seed oil contains at maturity approximately 7–10% gamma-linolenic acid (GLA, 18:3n6), an unusual fatty acid in plants but with proven value as a nutrient and pharmaceutical in humans (Horrobin, 1992).

Crops may be overwintered or spring-sown. Autumn sowing is the preferred option in the UK, where the start of post-winter growth occurs around mid-April and coincides with the onset of stem extension ("bolting") (Fieldsend and Morison, 2000). Formation of flower buds can be detected approximately one month later. For example, in 1992 flower buds were first identified in an overwintered crop of cv. Merlin by microscopic examination on 18 May, when

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the stems were between 0.3 and 0.4 m tall (Scotia, unpublished data). Flowering begins in late June and continues for about eight weeks. The period May–July is therefore a critical period for the crop, during which its potential seed yield is determined.

Most crop canopy photosynthesis occurs at light levels below those required to saturate photosynthesis (Baker et al., 1989) and any reduction in the efficiency of utilisation of absorbed light may lead to reduced crop productivity. Light energy absorbed by chlorophyll is either used to drive photosynthesis, lost as heat, or re-emitted as fluorescence. The ratio of variable to maximal chlorophyll fluorescence, Fv/Fm, can be used as an indicator of photosynthetic efficiency of plants in the field (Maxwell and Johnson, 2000).

In maize, a chilling-sensitive crop plant, low temperatures have been shown to detrimentally affect the development of the photosynthetic apparatus in developing leaves, leading to significant reductions in both the maximum quantum efficiency of CO_2 assimilation (ϕ_{CO2max}) and the light-saturated rate of CO_2 assimilation (A_{sat}) (Baker et al., 1989). Low temperature-dependent photoinhibition, defined as a light-induced reduction in the efficiency of CO_2, is a shorter-term phenomenon caused by the coincidence of low temperatures and high or even moderate light intensities (Farage and Long, 1991). In temperate areas, photoinhibition not only occurs in chilling-sensitive species, but has also been observed in more chilling-resistant species, including the C_3 crops oilseed rape (Farage and Long, 1991) and wheat (Groom and Baker, 1992).

In this study the effects of low temperature on photosynthesis in evening primrose leaves were examined. Firstly, the photosynthetic capacity of leaves which developed during May, June and July were compared by measuring their CO_2 assimilation. Secondly, in controlled environment studies and in the field, short-term changes in photosynthetic efficiency were measured in plants exposed to low temperatures in conjunction with high light intensities. Finally, a clear correlation between Fv/Fm and CO_2 assimilation was demonstrated.

Materials and methods

Plant material – glasshouse

The evening primrose cultivars Rigel, Merlin and Peter and the breeding lines PBC and LQB, all from the breeding programme of Scotia Pharmaceuticals Ltd., Stirling, UK, were used. Seeds were sown under glass in a peat-based compost (Levington M2, Fisons, Suffolk, UK) on 4 May 1994. The plants were potted up into 130 mm diameter pots on 22 June and were watered daily. These plants were used for the controlled environment studies.

Plant material – field

To simulate an autumn-sown crop, seeds of the five breeding lines were sown under glass in a peat-based compost on 19 January 1994. Seedlings were pricked out into modules and the plants were moved outside to harden off on 15 April, prior to transplanting into an experimental plot on the campus of the University of Essex, Colchester, UK (51°52'N, 0°57'E) on 9 May. Plant growth was apparent within days of transplanting.

The soil was a sandy silt loam of pH 4.8, P index 3, K index 3, Mg index 2, to which fertiliser (20:10:10, NPK, John Parsons Ltd., Boston, UK) was applied at a rate of 100 kg ha^{-1}. Glufosinate-ammonium herbicide was applied pre-planting to control weeds and subsequent weed control was by hand. The plant population density was 28 plants m^{-2} and the trial design was a randomised complete block, with five replications and two plants per plot. Air temperature and light intensity were recorded at one minute intervals by a weather station (Delta-T Services Ltd., Burwell, Cambridge, UK) located adjacent to the experimental plot and half-hourly means were calculated.

For gas exchange studies, a block of cv. Rigel plants in 130 mm diameter pots was planted out on the same date, such that the pot was completely buried in the ground. A population density of 28 plants m^{-2} was again used. The field trial and the block of plants in pots were each bordered by two rows of cv. Rigel plants transplanted directly into the ground at the same population density.

Measurement of CO_2 assimilation

A leaf disc from the upper region of a selected leaf was cut under water and, after removal of surface water, was placed in a leaf disc chamber of a design described by Stirling et al. (1991). Cut surfaces were continuously irrigated by circulating water which maintained leaf temperature at 19.5±1°C. The chamber was illuminated with a 250 W quartz-iodide source (Scholly Fiberoptik GmbH, Germany) fitted with a heat-reflecting filter (OCLI Ltd., High Wycombe, UK) and Q at the leaf surface was regulated by attenuating the beam with a series of neutral density filters constructed of muslin. A quantum sensor (SKP 200, Skye Instruments Ltd., Powys, UK) was used to measure Q incident at the leaf surface. The CO_2 gradient across the chamber was determined with an infrared CO_2 analyser (Type 225-Mk 3, Analytical Development Co., Hoddesdon, UK) calibrated using bottled air with a CO_2 concentration of 378 ppm (Cryoservice Ltd., Worcester, UK).

The controlled environment

The controlled environment consisted of a thermostatically controlled chest freezer pre-set to the desired temperature of 9, 13 or 27°C. The freezer lid consisted mainly of a sheet of Pilkington K glass (Pilkington Ltd., UK), which reduced infrared radiation transmission into the freezer to a minimum. The light source was a 400 W sodium vapour lamp (Thorn EMI Ltd, Ruislip, UK), positioned just above the freezer lid and directly above the eight leaves used for the study. The maximum light intensity (Q) at the leaf surface, as measured by a quantum sensor (Li-185B, Li-Cor Inc. Lincoln, Neb. USA), was 1100 μmol m^{-2} s^{-1}. When required, Q was reduced to 570 μmol m^{-2} s^{-1} using a neutral density filter.

Measurement of Fv/Fm

Fluorescence parameters were recorded using a portable fluorimeter (Plant Efficiency Analyser, Hansatech, Kings Lynn, UK) which consisted of a sensor unit attached by a cable to a control box. Selected, healthy leaves were first 'dark-adapted' by covering with a lightweight, white plastic leafclip fitted with a shutter. After five minutes dark adaptation the sensor unit was placed over the leafclip and the shutter plate pulled back so that the leaf was exposed to a five-second period of high intensity (3000 μmol m^{-2} s^{-1}) illumination of a peak wavelength of 650 nm. Initial (Fo), maximal (Fm) and variable (Fv, i.e. Fm – Fo) fluorescence and the ratio Fv/Fm were automatically calculated and displayed by the fluorimeter.

Results

Light response curves from field-grown plants

Five leaves, each from a different plant, were sampled on each of three occasions, 15–19 DAT (rosettes), 44–45 DAT (bolting, flower buds visible) and 75–80 DAT (flowering ongoing, well-developed seed capsules present). Initially the youngest, fully expanded leaves were excised under water from potted plants

brought from the field into the laboratory. By late July, the potted plants were experiencing water stress, so plants in the border rows were used and leaves were excised under water in the field. The leaves immediately below the reproductive part of the stem were selected. Assimilation rates were calculated using the equations of Coombs et al. (1987).

The parameters of the light response curves (ϕ_{CO2max} and A_{sat}) remained constant throughout the experiment (Fig. 1). CO_2 assimilation increased linearly (Fig. 1, insets) at light intensities between 50 and 200 μmol m^{-2} s^{-1}.

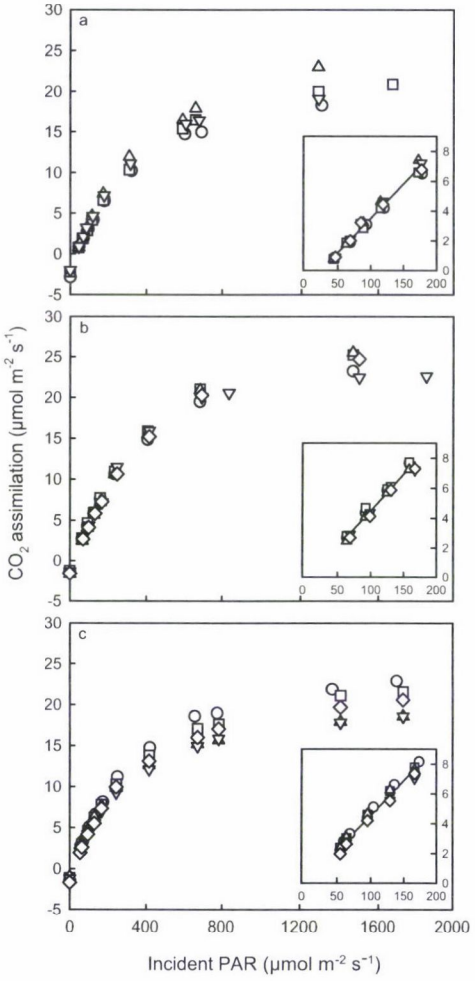

Fig. 1. Light response curves of net CO_2 assimilation per unit leaf area for healthy, fully expanded leaves of evening primrose sampled on (a) 24–27 May, (b) 2–22 June and (c) 22–27 July 1994 from a field plot in north-east Essex. Different symbols are used to distinguish the response curve of each leaf. Insets: ϕ_{CO2} (the gradient of the line of best fit) were (a) 0.046, (b) 0.048 and (c) 0.046

Variation in Fv/Fm under controlled conditions

In each controlled environment study eight plants at the rosette stage were moved from the glasshouse to the laboratory at 08.30 BST, when Q in the glasshouse, as measured by a quantum sensor (Li-185B, Li-Cor Inc.), was 100–200 μmol m^{-2} s^{-1}. Fluorescence parameters were recorded, then the plants were placed in the freezer with the selected leaf of each plant lightly supported in a horizontal plane and pointing to the centre of the illuminated zone. Care was taken to ensure that none of these leaves were shaded. Fv/Fm after five minutes of dark adaptation was measured at hourly intervals during the treatment period. The plants were removed from the freezer immediately after the last set of measurements, and recovery of Fv/Fm was measured at room temperature and lighting (approximately 28°C and 2.5μmol m^{-2} s^{-1}, respectively), initially at 15-minute intervals and subsequently at 30-minute intervals.

Effect of temperature on Fv/Fm

Plants of cv. Rigel were exposed to a light intensity of 1100 μmol m^{-2} s^{-1} for six hours at a temperature of either 9, 13 or 27°C. Fv/Fm declined from an initial value of 0.84 in all treatments with the greatest reduction being recorded at 9°C (where Fv/Fm was still dropping after six hours) and the smallest at 27°C (Fig. 2a). After the plants were returned to room conditions Fv/Fm rapidly recovered, although 2.5 hours after exposure to 9°C Fv/Fm was still only 0.80 and the rate of increase had slowed.

Effect of light level on Fv/Fm

Plants of cv. Rigel were exposed to light intensities of either 0, 570 or 1100 μmol m^{-2} s^{-1} for up to six hours at a temperature of 13°C. The greatest reduction in Fv/Fm was recorded in plants exposed to the highest light intensity (Fig. 2b), but Fv/Fm immediately started to recover upon return to room conditions. Recovery was complete after 24 h (data not shown). In plants not exposed to light, Fv/Fm had increased to 0.87 after one hour and then remained constant. Fv/Fm declined to 0.857 after the plants were returned to room conditions.

Differences in Fv/Fm response between cultivars

Four breeding lines were compared at a light intensity of 1100 μmol m^{-2} s^{-1} and a temperature of 13°C. The performance of cv. Merlin was similar to cv. Rigel (data not shown but c.f. Fig. 2a). From similar initial values (approximately 0.84) Fv/Fm declined in all lines, but the decline was greatest in LQB and least in PBC (Fig. 2c). Fv/Fm began to recover in all lines upon return to room conditions, but in LQB had only reached 0.78 after 3 hours.

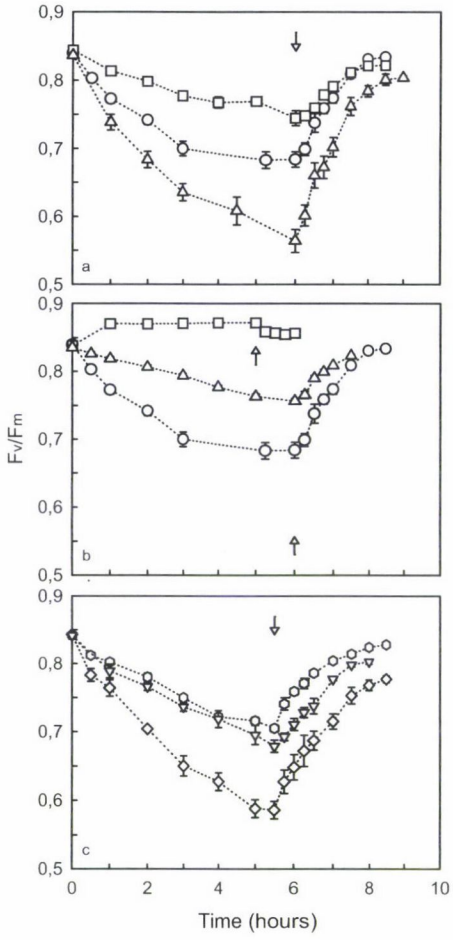

Fig. 2. Time course of Fv/Fm in attached leaves of evening primrose (a) cv. Rigel at temperatures of 27°C (squares), 13°C (circles) and 9°C (triangles) and a light intensity of 1100 μmol m^{-2} s^{-1}, (b) cv. Rigel at light intensities of 0 (squares), 570 (triangles) and 1100 μmol m^{-2} s^{-1} and a temperature of 13°C and (c) cv. Merlin (triangles) and breeding lines PBC (hexagons) and LQB (diamonds) at a light intensity of 1100 μmol m^{-2} s^{-1} and a temperature of 13°C. The arrows denote termination of treatment whereupon the plants were transferred to room temperature and lighting (approximately 28°C and 2.5 μmol m^{-2} s^{-1}, respectively). The error bars denote ±1 SE

Variation in Fv/Fm in the field

Fluorescence parameters of intact, disease-free leaves were recorded at 08.30 BST over the period 10–25 DAT using the portable fluorimeter after five minutes dark-adaptation. One measurement was made per plot on each occasion, giving five readings for each of the five cultivars. The time lapse between the application of the first and last leafclips was 13 minutes.

Significant changes in Fv/Fm occurred during this study (Fig. 3a). From 12 to 14 DAT relatively high (10–12°C) dawn temperatures (i.e. temperatures at 05.00 BST) were followed by dull days (Fig. 3b) and Fv/Fm values >0.8 were recorded in all lines. A gradual change to clearer skies then occurred and Fv/Fm values declined. Days 20 and 21 were sunny following slight overnight frosts and the lowest Fv/Fm values (0.6–0.7) were recorded on days 21 and 22. Subsequent days were also sunny, with the exception of day 24 which remained dull, but dawn temperatures were higher and Fv/Fm values again exceeded 0.8 on day 24. The low Fv/Fm values obtained on day 22 despite a dawn temperature of 6.6°C, are notable, as are the differences between breeding lines. The decline in Fv/Fm was fastest in LQB and slowest in PBC. The response patterns of cv. Merlin and cv. Peter (data not shown) were very similar to that of cv. Rigel.

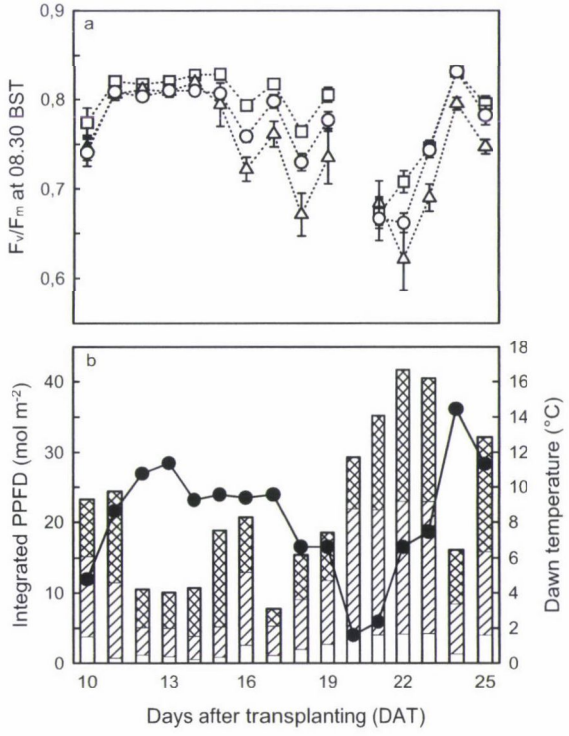

Fig. 3. (a) Fv/Fm at 08.30 BST in field-grown plants of evening primrose cv. Rigel (circles) and breeding lines PBC (squares) and LQB (triangles) on 19 May–3 June 1994. The error bars denote ±1SE. (b) Air temperature at 05.00 BST (closed circles) and cumulative integrated solar radiation (bars) for the periods 05.00–08.30 (lower, white), 08.30–12.30 (middle, hatched) and 12.30–16.00 BST (upper, opposite hatched)

Relationship between Fv/Fm and CO_2 assimilation

Fv/Fm was measured on a selected leaf of each of eight glasshouse-grown plants of cv. Peter, which were then placed in the freezer as previously described, with the selected leaves pointing to the centre of the illuminated area. The plants were exposed to a light intensity of 570 μmol m^{-2} s^{-1} and a temperature of 27°C for a minimum period of one hour. From one hour onwards, Fv/Fm was measured on each leaf just prior to its being excised under water and immediately transferred to the open gas exchange system. Here, the light intensity was 420 μmol m^{-2} s^{-1} and the rate of CO_2 assimilation stabilised within minutes, whereupon the leaf was removed from the chamber and Fv/Fm remeasured.

Assessments on eight leaves took approximately 80 minutes, following which the plants in the freezer were rotated to bring a further eight leaves, of comparable appearance to the first eight, into position under the light source. To maximise the reduction in Fv/Fm the freezer temperature was reduced to 9°C and the light intensity was increased to 1100 μmol m^{-2} s^{-1} for three hours, followed by a further hour at 9°C and 570 μmol m^{-2} s^{-1}. Fv/Fm and CO_2 assimilation rate for each leaf were then measured as described above.

The mean initial Fv/Fm value (0.85) was similar to earlier studies. For the first eight leaves the mean Fv/Fm value was 0.80 just prior to gas exchange analysis and 0.79 immediately afterwards. CO_2 assimilation rates ranged from 5.53 to 11.35 μmol m^{-2} s^{-1} (Fig. 4). The mean Fv/Fm value of the second eight leaves after chilling was 0.51 just prior to gas exchange analysis and 0.60 immediately afterwards (when individual results ranged from 0.40 to 0.75), indicating that recovery was already beginning to occur. CO_2 assimilation rates in these leaves ranged from 0.10 to 7.31 μmol m^{-2} s^{-1}. The correlation ($y = 22.5x - 9.95$) between CO_2 assimilation and Fv/Fm was significant ($r^2 = 0.709$, $p < 0.001$).

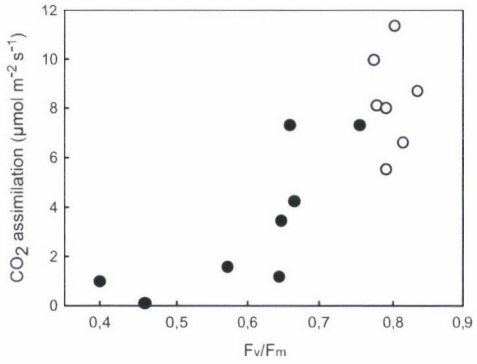

Fig. 4. Relationship between net CO_2 assimilation and Fv/Fm for evening primrose cv. Peter at temperatures of 27°C (open circles) and 9°C (closed circles) and a light intensity of 420 μmol m^{-2} s^{-1}.

Discussion

The values of the parameters of the light response curves in this study (Fig. 1) are comparable to those reported for other C_3 species. For example, if the proportion of the light incident on the leaf surface that is absorbed is assumed to be 83.8%, the mean absorptance for 37 C_3 species studied by Bjorkman and Demmig (1987), ϕ_{CO2max} in evening primrose is 0.056, very similar to the value obtained for oilseed rape by Farage and Long (1991). A_{sat} was typical of many C_3 plants. The intrinsic ability of evening primrose leaves to carry out photosynthesis was unaffected by changes in weather conditions that occurred immediately prior to, and during this study, suggesting that high photosynthetic rates can potentially be maintained in healthy leaves for long periods of the year.

In the controlled environment studies the Fv/Fm of unstressed leaves of all evening primrose breeding lines was approximately 0.84 (Fig. 2). In the field a similar result (0.831) was recorded in cv. Rigel on the morning of 2 June (24 DAT), a mild, dull day, and in all breeding lines Fv/Fm exceeded 0.80 for the period 11–15 DAT (Fig. 3). These values are similar to the mean value of 0.832 for healthy, unstressed leaves of several C_3 species reported by Bjorkman and Demmig (1987).

Although the Fv/Fm results under unstressed conditions were remarkably consistent between breeding lines, differences were shown to exist in susceptibility to low temperature-dependent photoinhibition. In most instances Fv/Fm recovered rapidly (substantially complete after 1 h, fully recovered after 24 h). Demmig-Adams (1990) quoted several field studies in which recovery of photosynthetic efficiency had been observed over a similar timescale and proposed that a regulated dissipation of the excess of excitation energy within the photochemical system via the xanthophyll cycle would normally, if not always, protect the photosystem II (PS II) reaction centres from damage. In contrast, the delayed recovery of Fv/Fm in LQB noted both under controlled conditions (Fig. 2c) and in the field (Fig. 3a) suggests that damage to the D1 protein in the reaction centre may have occurred, since this repair cycle can take days (e.g. Baker et al., 1989).

Although the correlation between Fv/Fm and CO_2 assimilation is evident at low and normal temperatures (Fig. 4), it seems that the inhibition of CO_2 assimilation at low temperatures is not only due to the inhibition of PS II activity but also to the inhibition of many metabolic processes that occur during CO_2 fixation.

Mid to late May is a time of rapid stem extension in overwintered crops in the UK and when flower primordia would be visible. Differences between cultivars in susceptibility to low temperature-dependent photoinhibition can occur during this period. These differences could affect the rate of biomass assimilation and possibly the yield potential of evening primrose in cool climates.

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USE OF SILVER THIOSULPHATE (STS) AND 1-METHYLCYCLOPROPENE (1-MCP) TO IMPROVE THE SHELF LIFE OF MINIATURE POTTED ROSE CV. AMORE

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The quality of miniature potted roses during their shelf life is limited by bud abscission and premature flower senescence. *Rosa hybrida* L. cv. Amore plants were pretreated with silver thiosulphate (STS) at 0.2 and 0.4 mM and with 1-methylcyclopropene (1-MCP) at 0.3, 0.5 and 0.7 g m^{-3} for 6 h in order to investigate the effects of these chemicals on the postharvest quality. Both chemicals extended the flower longevity as well as the plant display life compared with the untreated control. The best treatments in this respect were STS at 0.4 mM and 1-MCP at 0.5 g^{-3} for 6 h, which resulted in the least degradation in the chlorophyll content of the leaves. The treatment with STS at 0.4 mM increased the flower longevity and plant display life by 1 and 1.67 days, respectively, compared with the 1-MCP pretreatment at 0.5 g m^{-3}. Since 1-MCP treatment does not have the heavy metal implications of STS treatment, the use of 1-MCP pretreatment for extending the shelf life of miniature potted rose cv. Amore was recommended.

Key words: rose, *Rosa hybrida* L. cv. Amore, shelf life, silver thiosulphate, 1-methylcyclopropene

Introduction

The shelf life of many flowering potted plants, including potted roses, is limited by the loss of flowers, buds and sometimes leaves. These effects are often caused by ethylene, which may be endogenous or exogenous (Muller et al., 2001). Miniature roses are increasingly popular as flowering potted plants. The acceptability of miniature roses in the trade, and particularly for exporting to different countries, depends on adequate postharvest longevity and quality. Leaf yellowing and flower, bud and leaf abscission are important quality problems in potted rose plants during marketing. Bud, flower and leaf drop are known to be the result of ethylene action (Serek, 1993; Serek et al., 1994).

Because of the diverse and often harmful effects of ethylene on a wide range of plant species, it would be highly beneficial to eliminate the effect of ethylene during the shelf life of potted plants. The most common chemical used in the floral industry against ethylene is STS (silver thiosulphate). Serek (1993) reported that treatment with STS improved the keeping quality of potted roses. Bud abscission and flower senescence were decreased and the longevity of flowers and whole plants was improved by applying STS. Serek et al. (1994) found that potted roses treated with STS lasted longer than the control in an indoor environment. Tjosvold et al. (1994) mentioned that the spray application

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of 1 mM STS strongly promoted the flower display of potted miniature roses compared to the control. Serek et al. (1996) found that STS had dramatic effects in inhibiting the ethylene-stimulated abscission of buds, leaves and flowers of potted roses, regardless of the cultivar.

Ichimura et al. (1998) reported that although treatment with STS, an ethylene inhibitor, extended flower longevity, it contained silver, which is a potential environmental pollutant. Therefore, other ways of extending vase life may be necessary. Serrano et al. (2001) also reported that STS is a potential environmental hazard and noted that many countries currently prohibit its use.

A new tool, 1-methylcyclopropene (1-MCP), has been added to the list of options for extending the vase life of cut flowers and potted plants. 1-MCP was discovered by Prof. E. C. Sisler, of North Carolina State University, Raleigh, North Carolina, United States and is marketed under the trade name EthylBloc. 1-MCP is a gas in its natural state (as is ethylene), which provides both opportunities and challenges in commercial use. EthylBloc comes in powder form, which is added to water to release the gas.

Serek et al. (1994; 1996) found that potted roses treated with 1-MCP lasted ten days longer than the control in an indoor environment. The same effect was found for potted plants of begonia and kalanchoe. A dramatic inhibition in the deleterious effect of ethylene in these potted plants was noted when applying STS and 1-MCP. 1-MCP was at least as effective as the standard commercial treatment with STS for potted begonia plants (Serek, 1995). Muller et al. (1999) reported that pretreatment with 1-MCP delayed the flower senescence promoted by abscisic acid (ABA) in potted rose cv. Bronze, suggesting that the effect of ABA is at least partly mediated by ethylene. Pretreatment with 1-MCP before transport simulation improved the display life of potted roses, whether there was transport stress or not (Muller et al., 2000). Serek and Sisler (2001) and Celikel et al. (2002) concluded that 1-MCP will be an important tool for preventing the postharvest deterioration of buds and flowers caused by ethylene in campanula and lilies.

The aim of this study was to make a comparison between the effect of STS and 1-MCP on the postharvest quality of miniature potted rose cv. Amore and to probe their role in the chlorophyll content, which is connected to the longevity and quality of potted roses.

Materials and methods

Plant materials

Miniature rose plants (*Rosa hybrida* L. cv. Amore) produced in a commercial nursery in Hungary were used in this experiment. The plants were transported to the experimental greenhouse of the Ornamental Plants Department, Faculty of Horticultural Sciences, Budapest in the bud stage. The plants were grown in 10 cm pots and irrigated with tap water as needed. After 4 days the plants were at normal commercial maturity (2–4 open flowers) and were hence transferred to the indoor environment room to begin the treatments. Three replications were used per treatment in this experiment and the plants were arranged in a completely random block design. The experiment was repeated twice in 2003 and 2004.

1-MCP treatment

Treatment with 0.3, 0.5 and 0.7 g m^{-3} concentrations of 1-MCP was conducted at 19°C for 6 h. 1-MCP was obtained from the AgroFresh Inc. Rohm and Haas Company, Italy. The plants were placed in a 118×28×44 cm box sealed well with a plastic cover, and the required concentrations of 1-MCP, calculated as g m^{-3} EthylBloc powder, were placed in a test tube inside the box. Since a significant percentage of the 1-MCP is released immediately after the addition of hot water, the box was first sealed, and then hot water was injected into the test tube (just enough to cover the powder for each treatment).

STS treatment

STS solution was prepared as described by Gorin et al. (1985). The plants were sprayed with 0.2 and 0.4 mM concentrations of STS with the addition of 0.1% Tween 80 surfactant to the run-off point. The control plants were sprayed with tap water containing the same concentration of surfactant. Control and STS-treated plants were kept in a sealed box for 6 h as in the 1-MCP treatment, before being placed in the indoor environment room to evaluate the shelf life.

Flower longevity and shelf life determination

After the treatments, the plants were placed in the indoor environment room at 21 ± 1°C, 70–80% relative humidity (RH) and natural day-light (calculated for Budapest in June). The shelf life, longevity of individual flowers and percentage of healthy flowers per plant were evaluated.

Display quality was determined every second day by counting the number of healthy flowers on each plant. Healthy flowers were defined as not abscised, half-open and open flowers (at stages 2 or 3 in Fig. 1) without any senescence symptoms (Muller et al., 1998). The display life was defined as days until 50% of the flowers had faded. The longevity of individual flowers was defined as the number of days from the beginning of bud opening until stage 4.

Chlorophyll content of leaves

Leaf samples were taken on day 0 (at the beginning of the experiment), day 5 and at the end of display life in the control. The leaf chlorophyll content was extracted with acetone as previously described by Dawood (1993). Extraction in acetone was repeated until all the pigment had been extracted from the samples. The absorbance of the extracts was determined in a spectrophotometer (UV/VIS 916, GBC, Australia). The chlorophyll content was calculated as mg g^{-1} fresh weight. The equations for the determination of the concentrations of chl *a* and chl *b* were:

$$\text{chl } a = 11.24\, A_{661.6} - 2.04\, A_{644.8}$$

$$\text{chl } b = 20.13\, A_{644.8} - 4.19\, A_{661.6}$$

where *A* is absorbance.

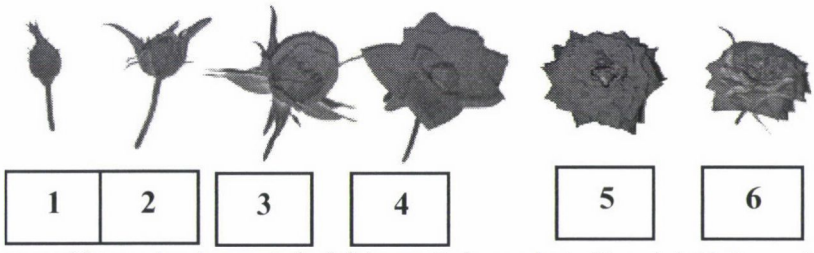

Fig. 1. Stages of flower development of miniature potted rose plants (*Rosa hybrida* L. cv. Amore) during shelf life. (1) Tight bud, (2) Half-open bud, (3) Open flower, (4) Incipient senescence, (5) Senescent flower and (6) Wilted flower.

Statistical analysis

The results were analysed using SPSS program Base 9, SPSS Inc., USA. The analysis of variance (ANOVA) and the differences between means were calculated using Duncan's multiple range test at the 0.05 level.

Results

Effect of STS on flower longevity and shelf life

Pretreatment with STS improved flower longevity and consequently extended the display life of potted rose plants (Fig. 2). Both concentrations of STS significantly increased these parameters compared with the control, but 0.4 mM STS treatment resulted in the best quality in this respect, with flower longevity and display life values of 19.33 and 22 days, respectively.

Effect of 1-MCP on flower longevity and shelf life

Treatment with 1-MCP had a beneficial effect on both the flower longevity and plant display life of potted roses. All the concentrations applied significantly extended the longevity of miniature potted roses compared with the untreated control (Fig. 2), the best results being achieved with 1-MCP at 0.5 g m^{-3} for 6 h (18.33 and 20.33 days for flower longevity and display life, respectively). However, treatment with 0.4 mM STS gave longer flower longevity and plant display life than any concentration of 1-MCP applied.

The STS and 1-MCP treatments showed great differences in display quality as measured by changes in the percentage of healthy flowers during the period of shelf life (Fig. 3). During the first 10 days of shelf life, the control plants lost quality rapidly and there were significant differences between the untreated control and all concentrations of STS and 1-MCP, while there were no differences between the two ethylene inhibitors used. By day 16, however, there were significant differences between STS and 1-MCP, treatment with 0.5 g m^{-3} 1-MCP for 6 h resulting in 58.33% healthy flowers, as opposed to 66.66% healthy flowers with 0.4 mM STS (Fig. 3). These differences increased at later dates. The effect of STS at 0.4 mM and 1-MCP at 0.5 g m^{-3} for 6 h compared with the untreated control is shown in Figure 4.

Effect of STS and 1-MCP on the chlorophyll content

The chlorophyll content of the leaves was positively affected by various concentrations of STS and 1-MCP (Table 1). Higher values of chlorophyll *a* and *b* were observed on day 5 and there were significant differences between all treatments and the untreated plants. Although there were no significant differences between STS at 0.4 mM and all concentrations of 1-MCP for chl.*a*, there were differences between the two chemicals for chl.*b*, the highest chl.*b* content (1.06 mg g^{-1} fresh weight) being recorded with 0.5 g m^{-3} 1-MCP for 6 h compared with the control and the other treatments (Table 1).

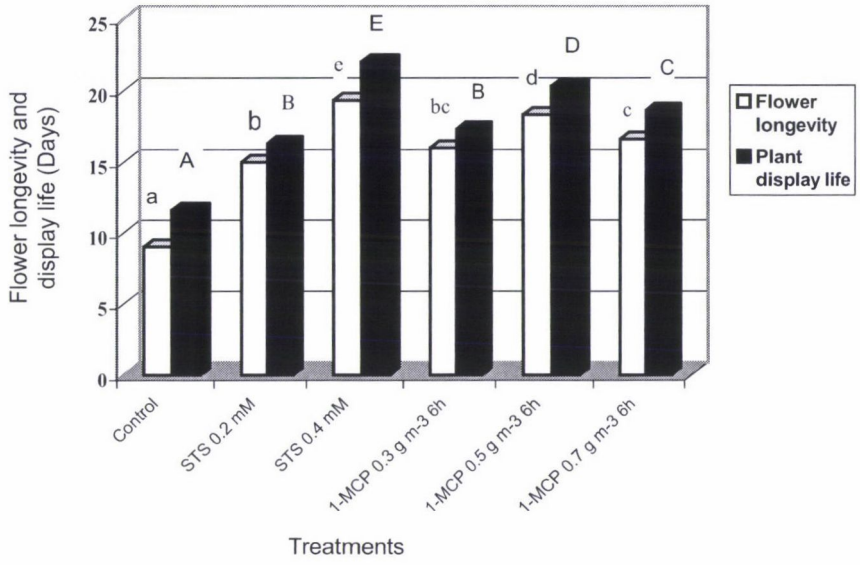

Fig. 2. Effect of STS and 1-MCP on the flower longevity and plant display life of miniature potted rose cv. Amore. Bars followed by different letters differ significantly from each other according to Duncan's multiple range test at $P = 0.05$

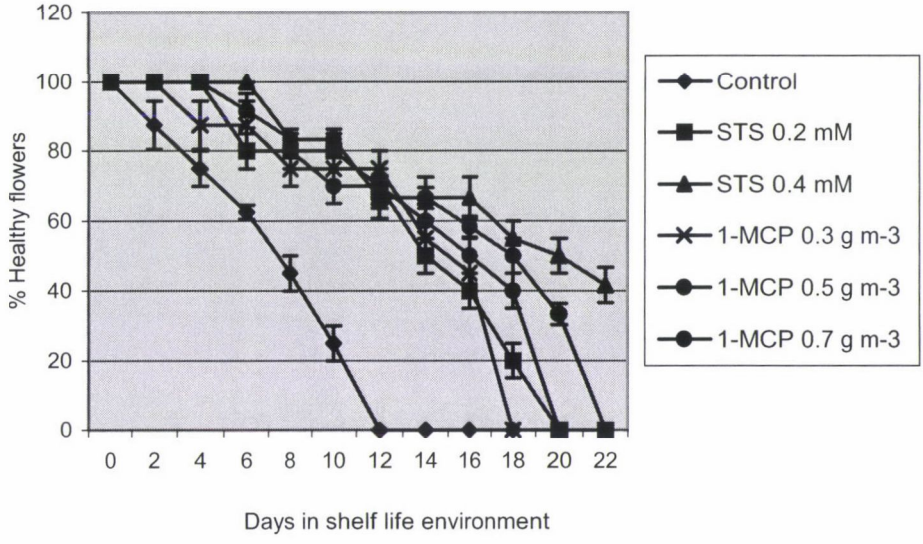

Fig. 3. Effect of different concentrations of STS and 1-MCP treatments on the longevity of miniature potted rose cv. Amore expressed as the percentage of healthy flowers in an ethylene-free environment compared with the untreated control during the 22-day observation period. Values are means of 3 replicates and each point represents mean ± S.D.

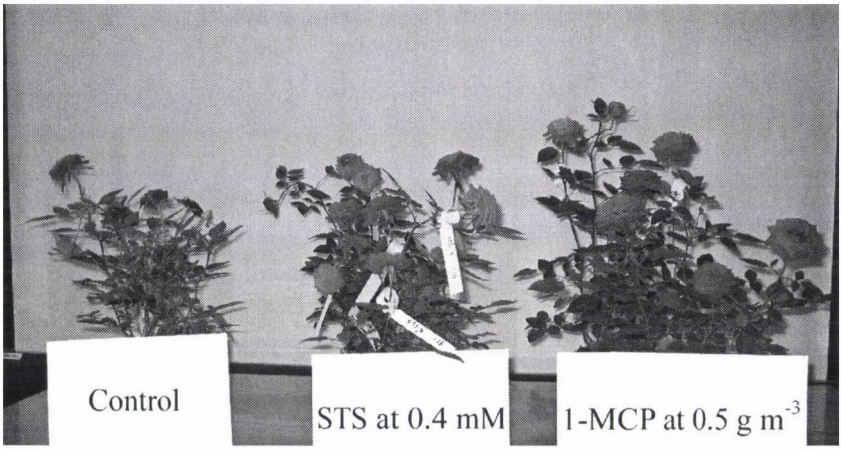

Fig. 4. Effect of 0.4 mM STS and 0.5 g m^{-3} 1-MCP for 6 h compared with the untreated control. During pretreatment, the STS-treated and control plants were placed in air in boxes identical to those used for the 1-MCP treatment. After pretreatment, all plants were transferred to the evaluation room. The photo was taken 10 days after transporting to the evaluation room

The chlorophyll content at the end of the display life of the control plants showed the beneficial role of STS and 1-MCP in retarding chlorophyll degradation in the leaves. All the concentrations of STS and 1-MCP significantly inhibited the chlorophyll degradation compared with the untreated control. The best results in this respect were obtained by the treatments with STS at 0.4 mM or 1-MCP at 0.5 g m^{-3}. In general, there were no significant differences between these two treatments (Table 1).

Table 1
Effect of STS and 1-MCP on the chlorophyll content (mg g^{-1} fresh weight) of leaves of miniature potted rose cv. Amore

Treatments	Time	Chlorophyll content	
		chl.*a*	chl.*b*
Initial content	Day 0	1.57 b	0.5 b
Control	Day 5	1.91 d	0.61 c
STS 0.2 mM	,,	2.48 e	0.78 e
STS 0.4 mM	,,	3.02 f	0.86 f
1-MCP 0.3 g m^{-3}	,,	2.96 f	0.96 g
1-MCP 0.5 g m^{-3}	,,	3.02 f	1.06 h
1-MCP 0.7 g m^{-3}	,,	2.96 f	0.87 f
Control	End *	0.87 a	0.33 a
STS 0.2 mM	,,	1.87 bc	0.55 b
STS 0.4 mM	,,	2.33 e	0.68 d
1-MCP 0.3 g m^{-3}	,,	1.93 d	0.77 e
1-MCP 0.5 g m^{-3}	,,	2.13 d	0.87 f
1-MCP 0.7 g m^{-3}	,,	1.78 bc	0.70 d

Means followed by different letters differ significantly from each other according to Duncan's multiple range test at $P = 0.05$ and statistical analysis is valid only within a column.
* End of the shelf life of the control plants.

Discussion

In this study the flower longevity and plant display life of miniature potted roses, expressed as the percentage of healthy flowers, were improved after pretreatment with STS or 1-MCP. Both compounds extended flower longevity and plant display life. The positive effect of STS may be due to its role in the inhibition of ethylene effects, leading to an increase in the percentage of healthy flowers. In addition, these results could be explained by the good quality of the leaves and the minimum chlorophyll loss obtained in this treatment (Table 1). Similar results were obtained by Tjosvold et al. (1994) and Serek et al. (1996). Muller et al. (2001) reported that ethylene production may be a major factor in determining the shelf life and quality of potted roses, so these ethylene inhibitors improved the shelf life.

The increase in the flower longevity and display life of potted roses achieved with 1-MCP may be due to its role in irreversibly binding to ethylene receptors (Serek, 1995; Serek et al., 1996). After pretreatment most of the receptors are blocked, thus inhibiting the ethylene action. These results were in harmony with the findings of Serek et al. (1994; 1996) and Muller et al. (1999). This treatment also kept the leaves turgid and inhibited chlorophyll loss (Table 1). Similar results were reported in previous studies with different ornamental crops (Serek et al., 1996; Celikel et al., 2002).

Treatment with 0.4 mM STS increased flower longevity and plant display life by 1 and 1.67 days more, respectively, than pretreatment with 0.5 g m^{-3} 1-MCP. Larger differences in the effectiveness of both ethylene inhibitors were observed in the indoor environment room in an ethylene-free atmosphere. Until the 10^{th} day of evaluation there were no significant differences between the two inhibitors, but increasingly large differences started to appear after this time and STS was found to be more effective than 1-MCP. These results are in agreement with the findings of Serek and Sisler (2001), who stated that 1-MCP molecules bind irreversibly to ethylene receptors so that after pretreatment most of the receptors are blocked. However, during further plant development new sites are synthesized and such receptors are not protected by 1-MCP. STS, on the other hand, remains in the plant tissues for a longer time and continuously inactivates ethylene responses after the synthesis of new sites. Reid et al. (1999) reported the same trend.

1-MCP treatment does not have the heavy metal implications of STS treatment, and there should be no waste disposal problem. Since the material is a gas its use would obviate the need for placing flowers in additional treatment solutions, which is labour-intensive. In addition, the use of STS, a possible environment pollutant, has been banned in several countries and 1-MCP is an effective and safe alternative to STS (Cross, 1996; Ichimura et al., 1998; Serrano et al., 2001). Consequently, 1-MCP pretreatment can be recommended for extending the shelf life of miniature potted rose cv. Amore.

Conclusions

It could be concluded that 1-MCP is an effective blocker of ethylene action in the miniature potted rose cv. Amore. Furthermore, its non-toxic character makes the material an excellent replacement for the environmentally unsafe silver ion.

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FIELD RESISTANCE OF MARTONVÁSÁR WINTER WHEAT CULTIVARS AGAINST FUSARIUM HEAD BLIGHT

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Fifty *Triticum aestivum* genotypes, including winter wheat cultivars from Martonvásár, were tested for fusarium head blight (FHB) resistance under artificially inoculated conditions. Field resistance, kernel infection, and the relative yield components (test weight, thousand kernel weight and kernel weight/heads) were examined following infection with *Fusarium graminearum* and *F. culmorum* isolates. Using data from two years, a number of Martonvásár varieties with above-average resistance to FHB were identified. On the basis of field infection, AUDPC values close to those of resistance sources were calculated for the variety Mv Emese, while 67.5% of the varieties tested had values which did not differ significantly from those of the control variety Arina. The yield components examined were modified substantially by artificial FHB infection. The thousand kernel weight and test weight of the variety exhibiting the greatest degree of infection were only 21.14% and 25.58%, respectively, of the untreated control. In one case the decline in the kernel weight/head was more than 90%. The results of multivariable statistical analysis indicated that among the Hungarian wheat genotypes, Bánkúti 1201, B9086-95 (a line derived from Bánkúti 1201), Mv Emese, Martonvásári 4 and Mv Táltos could be grouped with the best sources of resistance. The experimental data revealed wide genetic variability for FHB resistance in the Martonvásár breeding stock.

Key words: Fusarium head blight, wheat, field resistance, kernel infection, yield components

Introduction

From the beginning of the last century, fusarium head blight (FHB) received increasing attention as one of the most destructive diseases of wheat (Atanasoff, 1920; Husz, 1925). Among the *Fusarium* species causing head blight in Hungary the most important from the environmental (Parry et al., 1995), pathogenic (Mesterházy, 1977) and toxicological (Szécsi, 1990) points of view are *F. graminearum* and *F. culmorum*.

The two main types of resistance against FHB in wheat were described by Schroeder and Christensen (1963). Plants may have resistance either to fungal penetration into spike tissue (Type I), and/or to the spread of infection within the wheat head (Type II resistance). Among the various methods of artificial inoculation, spray inoculation proved to be the most efficient for the routine screening of FHB resistance in cereals. The results of these tests show the field resistance of the genotypes, resulting from the two main resistance types together (Miedaner et al., 2003).

A number of parameters can be used to determine the fusarium head blight resistance of wheat varieties. Spike infection can be estimated from the ratio of bleached spikelets (Snijders and Perkowski, 1990). Further information on the intensity of infection can be obtained by analysing threshed wheat kernels. As the result of infection with *Fusarium* species there is a decrease in the kernel number and kernel weight per head, and in the thousand kernel and test weight of the yield, while the damaged kernels are whitened, and in the case of high pathogen pressure and susceptible varieties the kernels are shrivelled and covered with mycelia. Szunics and Szunics (1981) studied the damage caused by *Fusarium* infection under natural conditions for a number of years and found that in the case of severe infection the thousand kernel weight of grains from diseased spikes of the variety Bezostaya 1 was 54.1% of that of healthy spikes, while the kernel weight per head was 43.7%. Mesterházy (1995) reported that the artificial inoculation of a susceptible variety led to a reduction in the yield to as little as 22.75%, with a kernel infection rate of 69.04%. According to Buerstmayr et al. (1999) there were great differences in the test weights of *Fusarium*-infected samples, ranging from 25 g to more than 37 g/50ml.

Only a limited number of FHB resistant varieties are currently available to breeders, so intensive work is in progress worldwide to find new resistance sources. At present spring genotypes of Far-Eastern origin, especially Sumai 3 and its derivatives (e.g. CM82036), are considered to have the best resistance (Bai and Shaner, 2004), but the agronomic traits of these genotypes differ greatly from those of the winter wheat varieties cultivated in Hungary. The same is true of spring varieties from Brazil (e.g. Frontana). Many winter wheat varieties bred in Europe and claimed in the literature to be resistant, proved in later experiments to be only moderately resistant (e.g. Arina; Ruckenbauer et al., 2001) or to have Type II resistance (e.g. F201*R*; Shen et al., 2003), which does not provide sufficient protection against attacks by the pathogen in the case of severe FHB epidemics (Argyris et al., 2003). Mesterházy et al. (2004) suggested that genotypes not derived from the known resistance sources should be screened as a possible way of broadening genetic variability. According to Liu and Wang (1991), instead of using Chinese varieties with excellent resistance for FHB resistance breeding, it would be expedient to use varieties with moderate resistance, but excellent agronomic properties, since genotypes with very good FHB resistance could well be found among the progeny as the result of transgressive segregation.

Research on fusarium head blight has been underway at the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár since 1972 (Szunics et al., 1987). New methods of testing varieties and advanced lines were introduced in 1999, when irrigation equipment was installed in the artificially inoculated nursery. Methodological experiments over the last five years have led to the optimisation of infection conditions and the selection of the *Fusarium* isolates best suited to local conditions. During the 2002/2003 vegetation season routine tests were begun on Martonvásár lines and varieties, aimed at appraising the level of FHB resistance in the Martonvásár breeding stock and, possibly, identifying new sources of resistance. The results of this work are presented in this paper.

Materials and methods

Plant material

The FHB resistance of 50 *Triticum aestivum* genotypes, including 40 Martonvásár winter wheat varieties, was tested under artificially inoculated conditions in the 2002/2003 and 2003/2004 vegetation seasons. Varieties known to be resistant (CM82036, Goldfield), moderately resistant (Nobeoka bozu, Arina) or susceptible (GK Zugoly) to FHB were included as controls (Buerstmayr et al., 1996; Ohm et al., 2000). Based on the results of earlier experiments, the variety Bánkúti 1201, which has above-average resistance, and two lines selected from the population of this variety (B9086-95, B9158-95) were also tested. The genotypes were sown in autumn in plots measuring 2 m^2 in the artificially inoculated FHB nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences.

Inoculum production

Isolates from two *Fusarium* species were multiplied in the laboratory for use as inoculum. The *F. graminearum* isolate 'IFA-65' was cultured in liquid mung bean medium (Buerstmayr et al., 2002), while the *F. culmorum* isolate 'IFA-104' was cultured on a 3:1 (w/w) mixture of autoclaved wheat and oat seeds and incubated in the dark for 2 weeks at 25°C, followed by 3 weeks at 5°C (Snijders and Van Eeuwijk, 1991). Prior to the inoculation of wheat heads, the conidia were washed off the kernels, and the concentration of the spore suspension was determined for both isolates and adjusted to 5×10^4 conidia/ml.

Field experiment

When the plants were at the 50% flowering stage, groups of main spikes on each plot were tied into bunches. The complete surface of the spikes in some of these bunches was sprayed with conidium suspensions of *Fusarium graminearum* or *F. culmorum*, in three replications, while the control bunches were left untreated. The treatment was repeated 2 days later. To ensure high humidity for the penetration and spread of the fungus, a mist irrigation system was operated during the trial.

Following infection, the ratio of spikelets killed by the FHB infection was determined every four days from the 14^{th} to the 26^{th} day. These data were used to calculate the area under the disease progress curve (AUDPC). At full maturity ten spikes were collected from each bunch, in all three replications, and the threshed kernels were used for measurements on kernel weight, test weight, thousand kernel weight and the ratio of shrivelled kernels covered with mycelia (kernel infection). For the first three of these traits statistical analysis was based on values relative to the untreated control, while in the case of kernel infection the actual values were used. The data were analysed using analysis of variance, after which the FHB resistance of the wheat varieties was examined using cluster analysis.

Results

The size of the area under the disease progress curve (AUDPC) was calculated for each variety after scoring the spikes for infection in the field 14, 18, 22 and 26 days after spraying. The results were evaluated according to Sváb (1981), using analysis of variance for the results of multifactorial experiments (Table 1).

The ANOVA results indicated that in the present experiments only the variety had a significant effect. Averaged over the two isolates and the varieties, the mean AUDPC values were 644.96 in 2003 and 673.09 in 2004. Averaged over years and varieties, the AUDPC values were 616.30 for plots inoculated

with *F. graminearum* and 701.74 for those inoculated with *F. culmorum*. The distribution of the tested wheat varieties on the basis of AUDPC values can be seen in Figure 1, which also indicates the groups to which the control varieties belonged.

The distribution of FHB resistance in the tested wheat varieties can be satisfactorily described using a normal distribution function, as confirmed by the results of the Kolmogorov-Smirnov test (D_n=0.106).

Based on the mean AUDPC values, the degree of infection was not significantly different from zero for five wheat varieties. Four of these (CM82036, Goldfield and the two lines of Bánkúti 1201 origin) were previously known to have excellent resistance to FHB. The variety Mv Emese was grouped with these genotypes, as it had an AUDPC value of 173.5, averaged over two years and two isolates. On the basis of field infection, a substantial proportion (67.5%) of the Martonvásár varieties had FHB resistance as good as that of Arina, which is considered as a source of resistance. It should be noted, however, that in the present experiments Arina was classified in the moderately resistant group on the basis of its AUDPC value (567.5). The degree of infection in four wheat varieties did not differ significantly from that of the susceptible control, GK Zugoly, and only 12 or 15 varieties exhibited significantly greater spike infection that Arina and Nobeoka bozu, respectively.

The data on kernel infection and yield reductions in 2003 were then analysed using analysis of variance. The results of this analysis are presented in Table 2, while Table 3 contains descriptive statistics on the parameters observed.

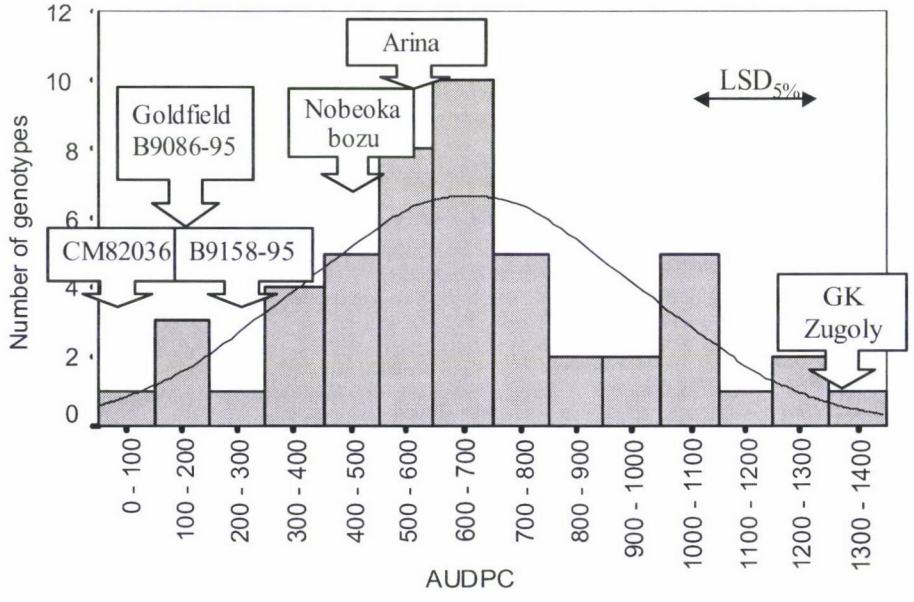

Fig. 1. Distribution of tested wheat varieties on the basis of FHB resistance

Table 1
Analysis of variance on the AUDPC values calculated from field FHB infection figures Martonvásár, 2003–2004

Factor	SQ	FG	MQ	F value
Total	25876629	199		
Year	39565	1	39565	
Isolate	364914	1	364914	
Isolate × year	3093828	1	3093828	
Variety	17444049	49	356001	13.03***
Variety × year	2136816	49	43608	
Variety × isolate	1458270	49	29761	
Variety × isolate × year	1339185	49	27330	

Note: *** = F value significant at the P=0.001 level

Table 2
F values of analysis of variance on kernel infection and yield reduction data, Martonvásár, 2003

Factor	Kernel weight/head (%)[a]	Test weight (%)[a]	Thousand kernel weight (%)[a]	Kernel infection (%)
Treatment	15.3***	9.3***	17.7***	24.4***
Variety	28.9***	17.1***	33.9***	47.4***
Isolate	43.1***	37.5***	2.6	0.4
Variety × isolate	1.1	0.9	1.7***	1.8***

Note: *** = F values significant at the P=0.001 level; [a]Relative values compared with the uninfected control

Table 3
Descriptive statistics for kernel infection and yield reduction data

Parameter	Minimum	Maximum	Mean	Standard deviation
Kernel weight/head (%)*	8.71	82.35	35.06	15.38
Test weight (%)*	25.57	89.21	54.99	13.47
Thousand kernel weight (%)*	21.14	83.33	46.28	14.16
Kernel infection (%)	17.00	97.67	69.81	19.95

*Relative values compared with the uninfected control

For each of the parameters the treatment effect and the variety effect were significant. The relative yield loss and the reduction in test weight depended significantly on the isolate used for inoculation, while the variety × isolate interaction was significant for the decrease in thousand kernel weight and for kernel infection, indicating that the effect of the isolate on these two traits depended on the variety. In susceptible varieties there was a substantial reduction in yield as the result of FHB infection. Averaged over the *Fusarium* species, the values obtained for test weight, thousand kernel weight and kernel weight/head in varieties exhibiting the greatest degree of infection were only 25.57%, 21.14% and 8.71%, respectively, of those recorded for the untreated control. The data were least affected by infection for line CM82036. With a

single exception (the reduction in thousand kernel weight in the variety Bánkúti 1201) infection caused a significant reduction in mass for all the parameters in all varieties, compared with CM82036. The results obtained for test weight, thousand kernel weight and kernel weight/head were not significantly different from or better than those of the moderately resistant variety Nobeoka bozu for 21, 9 and 9 Martonvásár wheat genotypes, respectively.

The mean kernel infection values obtained in the experiments were 69.55% for *Fusarium culmorum* and 70.07% for *F. graminearum*. The ANOVA results indicated that the effect of the isolates on this parameter was not statistically significant. Sixteen varieties exhibited kernel infection similar to that of the susceptible control, GK Zugoly, while for three varieties it was significantly more severe. The analysis demonstrated that the resistance of 6 Martonvásár varieties against kernel infection was statistically similar to that of the control variety Nobeoka bozu, while 8 were more resistant. Among these, the kernel infection of Martonvásári 4 did not differ significantly from that of Goldfield, the variety exhibiting the lowest level of infection.

Using the 2003 kernel infection and yield reduction data, together with the AUDPC values, the test varieties and control varieties were grouped by means of cluster analysis in an attempt to identify wheat varieties with FHB resistance similar to that of efficient resistance sources. After analysis using various methods, a group of 7 varieties was clearly distinguished, which included not only recognised sources of resistance (CM82036, Goldfield), but also Bánkúti 1201 and the line B9086-95 selected from it, together with three Martonvásár winter wheat varieties, Mv Emese, Mv Táltos and Martonvásári 4. The data obtained for these varieties are listed in Table 4, which also contains the relevant data for varieties with known resistance and for the susceptible control.

Table 4

AUDPC values of FHB-resistant Martonvásár varieties and control genotypes, and data on kernel infection and yield reductions averaged over the *Fusarium* isolates, Martonvásár, 2003

Genotype	AUDPC	Kernel infection (%)	Test weight (%)*	Thousand kernel weight (%)*	Kernel weight /head (%)*
CM82036	19.00	23.33	89.21	83.33	82.35
Goldfield	118.50	17.00	74.20	74.15	65.71
Mv Emese	119.00	43.33	70.08	70.33	56.53
B9086-95	188.50	51.67	74.82	63.95	58.72
Martonvásári 4	322.50	21.67	73.08	65.46	60.02
Mv Táltos	156.00	45.00	68.03	68.25	51.30
Bánkúti 1201	255.00	35.00	70.67	78.09	60.97
Nobeoka bozu	610.00	60.00	65.48	59.98	51.73
Arina	607.50	71.67	63.83	55.19	43.33
GK Zugoly	1545.00	85.00	38.36	26.21	16.63
$LSD_{5\%}$	233.45	8.08	9.09	6.78	7.98

*Relative values compared with the uninfected control

Discussion

In field experiments a method for testing FHB resistance was successfully adapted to local conditions, allowing reliable infection of approximately identical severity to be induced regardless of the year. This is confirmed by the results of analysis of variance (Table 1), since the F values for the year, the isolates and all the interactions were below the statistically significant level. This was achieved in two consecutive years with quite different levels of natural precipitation, the quantities of snow and rain in the vegetation period amounting to 250.6 mm in 2003 and to 583.2 mm in 2004.

According to data provided by the National Plant Protection Organization (Aponyi et al., 1998), FHB caused epidemics in Hungary in 9 out of 27 years. However, no data are available on the stage of development in which the pathogen most frequently attacked the plants. Infection during flowering, which has the most serious effect on yield quantity and quality, is probably much less frequent, since the weather during this critical period is not favourable for the pathogen in most years. Under such conditions, the sowing of varieties with good or moderate resistance, equal to or better than that of Arina, could lead to a substantial reduction in the risks facing wheat producers. The two-year field experiments indicated that a number of Martonvásár varieties come into this category. As approximately half of the 1.0–1.2 million hectare wheat-growing area in Hungary is sown to Martonvásár varieties, the FHB resistance of these varieties is of great economic significance.

A large number of new combinations have been developed in recent years using varieties with favourable agronomic and technological quality traits, which are also resistant or moderately resistant to fusarium head blight. According to Liu and Wang (1991), there is a good chance of selecting progeny with better traits than the parents from these combinations, as the result of transgressive segregation.

The present experiments confirmed earlier results on the outstanding resistance of lines selected from the old Hungarian wheat variety Bánkúti 1201. The agronomic properties of these lines differ considerably from those of the varieties cultivated today, but due to their adequate winter hardiness and excellent breadmaking quality they can be used as valuable sources of resistance in breeding.

The present paper contains the first results achieved with the modified testing method. The experiments are still in progress, but these results are already sufficient to indicate that the Martonvásár breeding stock contains wide genetic variability for FHB resistance.

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COMBINING ABILITIES OF CERTAIN CHARACTERS AND ESTIMATION OF HYBRID VIGOUR IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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This research was carried out in 1997 and 1998 in order to study the genetic structure of a hybrid population established from three CMS (cytoplasmic male sterile) lines and four pollen tester (restorer) lines, to identify the parents and crosses showing superior general and specific combining abilities and finally to evaluate F_1 hybrid vigour. According to the results, the variance due to specific combining ability (SCA) was highly significant for seed yield, number of seeds per head and plant height. These traits of sunflower were influenced, mostly, by dominant gene actions. Neither general (GCA) nor specific combining ability (SCA) variances were found to be significant for head diameter and 1000-seed weight. Most of the total genetic variation in these characteristics was caused by epistatic gene actions due to SCA variances, which were higher than GCA variances. The parental lines CMS381, CMS461, RHA684 and RHA892 had the highest positive GCA effects for seed yield and in terms of the other traits studied, but these effects were not significant for all the traits observed. The crosses CMS191×RHA723, CMS191×RHA892, CMS381×RHA684 and CMS461×RHA684 might be considered as promising combinations in terms of seed yield and yield components. The amounts of heterosis and heterobeltiosis ranged from –8.4 to +16.3% to –21.3 to +3.4% for plant height, from 46.3–82.3% to 20.3–48.3% for head diameter, from –14.8 to +52.6% to.–16.5 +46.9% for number of seeds per head, from –3.3 to +42.7 to –19.0 to +21.0% for 1000-seed weight and from 19.8–98.1 to 4.6–89.8% for seed yield.

Key words:.sunflower, *Helianthus annuus* L., combining ability, heterosis, line × tester

Introduction

Sunflower (*Helianthus annuus* L.) is a major oil crop in the drought regions of Turkey, where it is grown on around 0.5 million ha per year with average seed yields of 1.2–1.4 t ha^{-1} (Anonymous, 2002). In the last 20 years, the seed production of sunflower in Turkey has greatly increased, since high-yielding, adapted hybrid cultivars have been sown in these areas. The hybrid cultivar use reached 95% in Turkey during this period because the cross-pollinated cultivars previously grown were not generally promising for enhancing yields and were not resistant to orobanche (*Orobanche cumana* Wallr.). The importance of hybrid breeding in sunflower has increased recently because of their higher seed yield compared with cross-pollinated varieties. Hybrid sunflowers are more stable, highly self-fertile and more uniform at maturity (Dedio and Enns, 1976; Seetharam, 1979). Resistance to diseases and

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orobanche has also increased the importance of hybrid varieties. The heterotic performance of a hybrid combination depends upon the combining abilities of its parents (Allard, 1960; Kadkol et al., 1984). Information on combining ability is needed to identify potentially superior parents and hybrids, and would also help to define the pattern of gene effects in the expression of quantitative traits (Goyal and Kumar, 1991). The term "general combining ability" (GCA) is used to determine the average performance of a line in a hybrid combination. The term "specific combining ability" (SCA) is used to determine certain combinations relatively better or worse than would be expected on the basis of the average performance of the lines involved (Sprague and Tatum, 1942). The differences in GCA are mainly due to additive genetic effects and higher order additive interactions, while the differences in SCA are attributed to non-additive dominance and other types of epistasis (Falconer, 1989).

Various researchers have studied general and specific combining ability variances for several traits in sunflower. Kovacik and Skaloud (1972), Setty and Singh (1977) and Kadkol et al. (1984) found that specific combining ability variances for yield were significant, whereas Sindagi et al. (1979) reported that GCA variances were more effective than SCA variances for seed yield.

Sunflower, being a cross-pollinated crop, offers scope for developing new, superior varieties through heterosis breeding (Singh et al., 1984). High heterosis for yield characters has been widely reported in sunflower. Gundaev (1970) indicated that studies conducted in the USSR showed that hybrids yielded as much as 50% more than existing cultivars. In the USA Fick and Zimmer (1976) reported that hybrids yielded up to twice as much as check cultivars. Considerable heterotic effects were reported for head diameter, 1000-seed weight, plant height and seed yield in sunflower (Putt, 1966; Kloczowski, 1975; Chaudhary and Anand, 1984; Kadkol et al., 1984; Singh et al., 1984).

The aim of this study was to determine combining abilities, heterotic performances and the nature of gene actions for parental lines of sunflower.

Materials and methods

The population used in the study was established using the Line × Tester crossing method. Three cytoplasmic pollen-sterile genotypes, CMS191, CMS381 and CMS461, were used as female parents (lines) and four restorer genotypes, RHA684, RHA691, RHA723 and RHA892, as male parents (testers). These parental materials were selected from different origins, representing the elite varieties commonly grown in the world, by the Field Crops Department, Faculty of Agriculture, Uludağ University in Bursa, Turkey.

The three male-sterile lines were crossed with each of the four restorer lines in 1997. Seven parents and 12 F1 hybrids were tested in a field trial with replication under Bursa conditions (latitude 40°2'N, longitude 29°1'E, altitude 155 m) in 1998. Bursa is located in the southern Marmara region, with average annual rainfall of 713 mm and 14.4°C mean monthly temperature. The total rainfall during the growing period of sunflower (March to August) made up to 37% of the annual precipitation. The soil was clayey, and low in fertility. Soil analysis indicated that the phosphorus and potassium levels were medium or high and the organic matter was low (1.2%). The nitrogen levels in the soils were also low for sunflower.

Plantings were made by hand on 26 March in 1997 and 8 April in 1998. Each plot consisted of four rows, 5 m long with 0.70 m between rows, resulting in a total plot area of 14 m^2. All the rows were thinned to 0.30 m between hills. Sixty kg N/ha was applied as ammonium nitrate prior to sowing and a further 60 kg N/ha was applied when the plants started budding. After planting, Linuron was sprayed at a rate of 0.20 ml/m^2 for weed control. Hand hoeing was done when necessary. Twenty plants were selected randomly from each of the F1 hybrids and parents in each plot for observations. Data were recorded on plant height (cm), head diameter (cm), number of seeds per head, 1000-seed weight (g) and seed yield (kg/ha).

All statistical analyses were performed using MSTAT-C (version 2.1, Michigan State University, 1991) and MINITAB (University of Texas at Austin) software. The experiment was designed in a randomized complete block with three replications. The significance of variation sources was determined at the 0.05 and 0.01 probability levels using the F-test (Steel and Torrie, 1980). Analysis of variance for combining ability was done according to the Line × Tester method, in which estimates of GCA variances (σ^2_{GCA}) and SCA variances (σ^2_{SCA}) were obtained as suggested by Singh and Chaudhary (1977). Heterosis was calculated as a percentage increase or decrease in the F1 mean over its better parent and mid-parents. Means and heterotic effects were tested by the least significant differences (LSD) test at the 0.05 and 0.01 levels. The significance of GCA and SCA effects was determined at the 0.05 and 0.01 levels using the t-test.

Results and discussion

Analysis of variance for combining abilities revealed that there were no significant differences in terms of the GCA effects of lines and testers for any of the traits measured. The interactions between lines and testers were significant for plant height, number of seeds per head and seed yield (Table 1).

The ratios of GCA:SCA variance were lower than 1 for all the characters studied. Thus, non-additive gene actions were more effective for all the characters. As the variances due to SCA were highly significant for seed yield, number of seeds per head and plant height, these characteristics were influenced by dominant gene actions. Neither general nor specific combining ability variances were significant for head diameter or 1000-seed weight. Most of the total genetic variation for these traits was caused by epistatic gene actions, since the SCA variances were higher than the GCA variances (Table 1). The estimates of non-additive gene actions for all the characters in this study were generally in agreement with the results reported by Dua and Yadava (1983), Kadkol et al. (1984) and Pathak et al. (1985).

Comparative analysis of the GCA effects of the parents is given in Table 2. Although the GCA effects of the parents were not significant in terms of all the traits observed, the female parent CMS381 and two male parents, RHA684 and RHA892, had positive, high GCA effects for seed yield. In addition, highly positive GCA effects were recorded for CMS381 and RHA723 for 1000-seed weight, CMS461 and RHA892 for number of seeds per head, CMS381 and RHA691 for head diameter, and CMS461 and RHA892 for plant height (Table 2).

Table 1
Analysis of variance (mean squares) for combining ability

Source	df	Plant height	Head diameter	No. of seeds/head	1000-seed weight	Seed yield
Lines	2	176.7	2.9	27876.0	182.9	1728.3
Testers	3	90.3	3.9	19159.6	27.7	1540.8
Lines × Testers	6	241.2**	2.7	115376.5*	69.6	2697.9**
Error	36	49.4	2.2	34777.7	40.1	143.7
Estimates of variance components						
GCA		–2.281	0.016	–1818.2	0.395	–21.2
SCA		63.930**	0.166	26866.3*	9.833	851.4**
GCA:SCA		0.035	0.095	0.0067	0.040	0.025

* , **: Significant at p=0.05 and p=0.01, respectively; df: Degrees of freedom

Table 2
Estimates of GCA effects and mean values (M) of lines and testers for five traits

Parents	Plant height (cm)		Head diameter (cm)		No. of seeds/head		1000–seed weight (g)		Seed yield (kg/ha)	
	M	GCA	M	GCA	M	GCA	M	GCA	M	GCA
Lines										
CMS191	134.0	0.54	10.9	–0.56	740.2	3.4	37.1	–0.78	1273	–1.5
CMS381	130.2	–4.07	11.8	0.42	645.3	–49.8	40.4	4.23	1214	12.7
CMS461	138.0	3.53	11.5	0.14	895.3	46.4	34.2	–3.45	1430	–11.2
Mean of lines	134.1		11.4		760.3		37.2		1305	
Testers										
RHA684	103.7	1.19	7.8	–0.23	845.2	14.60	28.4	0.64	1112	7.30
RHA691	77.7	–3.52	7.4	0.88	893.4	–19.54	23.1	–2.38	953	–18.44
RHA723	84.1	–1.44	6.6	–0.69	932.0	–51.28	23.8	1.78	1029	0.12
RHA892	91.7	3.77	8.7	0.04	770.8	56.22	27.8	–0.04	.995	11.02
Mean of testers	89.3		7.6		860.3		25.8		1020	
Standard errors										
S.E. (Lines)		4.48		0.47		98.05		2.41		14.99
S.E. (Testers)		5.18		0.54		113.2		2.78		17.31

Data on the means and SCA effects of twelve crosses for all the traits observed (Table 3) suggested that the crosses CMS381×RHA684, CMS191×RHA892 and CMS191×RHA723 gave the highest seed yields (2304, 2159 and 2057 kg/ha, respectively). These hybrids also had the highest significant SCA effects for seed yield. Dominant gene actions were more effective for higher yield in these crosses. The hybrid CMS191×RHA723 showed a positive significant SCA effect, while the combinations CMS191×RHA892 and CMS381×RHA684 had positive but insignificant SCA effects for number of seeds per head. The SCA effects were not significant for any of the crosses in terms of head diameter and 1000-seed weight. It was concluded that the crosses CMS191×RHA723, CMS191×RHA892 and CMS381×RHA684 were better recombinants for the characters studied because of their positive and significant SCA effects (Table 3).

Significant heterosis was observed for various of crosses in all the traits (Table 4). The range of heterosis was also wide for all the different characters, with values ranging from 19.8% to 98.1% for seed yield, –8.4% to 16.3% for plant height; 46.3% to 82.3% for head diameter; –14.8% to 52.6% for number of seeds per head and –3.3% to 42.7% for 1000-seed weight. All the crosses expressed heterosis over the mid-parent for seed yield, values of over 70% being recorded for the crosses CMS381×RHA684 (98.1%), CMS191×RHA892 (90.4%), CMS191×RHA723 (78.7%), CMS381×RHA723 (75.1%) and CMS381×RHA892 (73.9%).

Ten hybrids showed positive, significant heterobeltiosis for seed yield (Table 4). The crosses CMS381×RHA684 (89.8%), CMS191×RHA892 (69.6%), CMS381×RHA723 (61.8%) and CMS191×RHA723 (61.6%) showed more than 60% heterosis over the better parent in the cross for seed yield. Similarly, nine hybrids exhibited positive but not significant heterosis over the better parent for 1000 seed weight, ranging between 2.9% and 21.0% (Table 4). Six hybrids had positive, significant heterobeltiosis for number of seeds per head, ranging from 25.1% to 46.9%. All the crosses were found to be superior for head diameter. Nine crosses showed negative, significant heterosis over the better parent for plant height (Table 4). The highest heterobeltiosis for 1000-seed weight was observed for hybrid CMS461×RHA723 (21.0%), followed by CMS381×RHA684 (16.6%) and CMS191×RHA892 (14.5%). The crosses CMS191×RHA892 (46.9%), CMS461×RHA691 (43.0%) and CMS381×RHA691 (40.2%) exhibited more than 40% heterosis over the higher parent for number of seeds per head. The highest heterobeltiosis for head diameter was observed for hybrids CMS381×RHA691 (48.3%), CMS461×RHA691 (44.3%) and CMS191×RHA892 (41.3%).

Table 3
Mean values (M) and SCA effects of the crosses for five characters

Crosses	Plant height (cm)		Head diameter (cm)		No. of seeds/head		1000 seed weight (g)		Seed yield (kg/ha)	
	M	SCA	M	SCA	M	SCA	M	SCA	M	SCA
CMS191×RHA684	108.9	–10.73**	14.4	–0.05	888	–166.5	34.8	–4.43	1429	–46.61**
CMS191×RHA691	111.2	–3.76	14.1	–1.43	966	–55.0	38.8	2.65	1646	0.75
CMS191×RHA723	126.9	9.92**	14.7	0.78	1174	185.3*	38.2	–2.18	2057	23.30**
CMS191×RHA892	126.8	4.57	15.4	0.69	1133	36.2	42.5	3.97	2159	22.66**
CMS381×RHA684	125.8	10.77**	15.6	0.26	1057	55.6	47.0	2.79	2304	26.71**
CMS381×RHA691	111.0	0.75	17.5	1.05	806	–161.6	44.5	3.23	1669	–11.05
CMS381×RHA723	102.5	–9.85**	14.2	–0.71	1004	68.4	43.8	–1.52	1964	–0.10
CMS381×RHA892	115.9	–1.69	15.0	–0.60	1081	37.6	39.1	–4.47	1921	–15.26*
CMS461×RHA684	122.5	–0.06	14.9	–0.20	1209	110.9	38.2	1.65	1998	20.02
CMS461×RHA691	120.9	3.02	16.6	0.39	1280	216.6*	27.7	–5.87	1646	10.41
CMS461×RHA723	119.9	–0.06	14.5	–0.11	778	–253.7*	41.4	3.73	1496	–23.06**
CMS461×RHA892	122.3	–2.88	15.3	–0.07	1066	–73.8	36.4	0.52	1763	–7.33
Mean of crosses	117.9		15.2		1037		39.4		1837	
S.E.		4.06		0.85		107.6		3.65		6.92

*Significant at p=0.05; **: Significant at p=0.01

Table 4
Heterosis (H_t) and heterobeltiosis (H_b) values of the crosses for five characters

Crosses	Plant height (cm)		Head diameter(cm)		No. of seeds/head		1000-seed weight (g)		Seed yield (kg/ha)	
	H_t	H_b	H_t	H_b	H_t	H_b	H_t	H_b	H_t	H_b
CMS191×RHA684	–8.4*	–18.7**	54.0**	32.1**	12.0	5.1	6.2	–6.2	19.8*	12.2
CMS191×RHA691	5.0	–17.0**	54.1**	29.3**	18.2	8.1	28.9*	4.6	47.9**	29.3**
CMS191×RHA723	16.3**	–5.3	68.0**	34.8**	40.4**	25.9*	25.4	2.9	78.7**	61.6**
CMS191×RHA892	12.3**	–5.4	57.1**	41.3**	49.9**	46.9**	30.9*	14.5	90.4**	69.6**
CMS381×RHA684	7.6	–3.4	59.2**	32.2**	41.8**	25.1*	36.6**	16.6	98.1**	89.8**
CMS381×RHA691	6.8	–14.7**	82.3**	48.3**	4.7	–9.8	40.2**	10.1	54.0**	37.5**
CMS381×RHA723	–4.3	–21.3**	54.3**	20.3*	27.3*	7.7	36.4*	8.4	75.1**	61.8**
CMS381×RHA892	4.5	–10.9**	46.3**	27.1**	52.6**	40.2**	14.7	–3.2	73.9**	58.2**
CMS461×RHA684	1.4	–11.2**	54.4**	29.5**	38.9**	35.0**	22.0	11.7	57.2**	39.7**
CMS461×RHA691	12.1*	–12.4**	75.7**	44.3**	43.1**	43.0**	–3.3	–19.0	38.1**	15.1*
CMS461×RHA723	7.9	–13.1**	60.2**	26.1*	–14.8	–16.5	42.7**	21.0	21.7**	4.6
CMS461×RHA892	6.5	–11.4**	51.5**	33.0**	27.9*	19.0	17.4	6.4	45.4**	23.3**
Average	5.6	–12.1	59.7	33.2	28.5	19.1	24.8	5.6	58.3	41.9

*Significant at p=0.05; **: Significant at p=0.01

Singh et al. (1984) reported high heterosis for seed yield, ranging from 47% to 206%, with values of 5% to 55% for other yield components. Chaudhary and Anand (1984) also reported that heterosis percentages over the better parents were 69.89% for seed yield, and 66.23% and 64.65% for 1000-seed weight and head diameter, respectively. The estimates of heterosis and heterobeltiosis for yield and for the remaining traits observed in the present study were in agreement with those reported by Pathak et al. (1983), Chaudhary and Anand (1984), Vágvölgyi (1984), Singh et al. (1984), Reddy et al. (1985) and Gorbachenko (1986).

As a result, genotypes CMS191, CMS381, RHA684 and RHA892 were the parents involved in the six best-yielding crosses. Among these parents, CMS381, which possesses a considerable positive GCA effect, might be utilized as a good line in hybrid sunflower breeding programmes. Parents RHA684 and RHA892, with higher GCA estimates, might be used as testers as well. On the other hand, CMS191×RHA723, CMS191×RHA892 and CMS381×RHA684 might be considered as promising hybrid combinations for higher yield based on their yield, heterosis and heterobeltiosis values and SCA effects.

Conclusions

Most of the hybrids having high heterosis for the characters studied also exhibited high SCA effects, thereby confirming the importance of dominant gene effects for these traits. In the study, the three hybrids (CMS 191 × RHA 723, CMS 191 × RHA 892 and CMS 381 × RHA 684) having significant positive heterosis and SCA effects for seed yield involved parents with low

GCA × low GCA, low GCA × high GCA and high GCA × high GCA effects, respectively. The seed yields of these hybrids, however, were significantly influenced by dominant gene actions, due to the fact that SCA variances were higher than GCA variances in the total genetic variation.

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NUTRIENT ECONOMY THROUGH LAND CONFIGURATION AND RESIDUE MANAGEMENT IN A GREENGRAM (*PHASEOLUS RADIATUS* L.)–WHEAT (*TRITICUM AESTIVUM* L.) CROPPING SEQUENCE WITH LIMITED WATER SUPPLIES

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A field experiment on a greengram–wheat cropping sequence was carried out under limited water supply conditions in 1997–98 and 1998–99 at the farm of the Indian Agricultural Research Institute, New Delhi. The greengram was sown either on flat beds or on broad beds 2 m in width, divided by furrows, with 0, 30 and 60 kg P_2O_5/ha. After the harvest of greengram pods, wheat was grown in the same plots, either with the greengram stover removed or with the stover incorporated along with 0, 40, 80 and 120 kg N/ha applied to wheat. The grain yield of greengram was higher when sown on broad beds with furrows compared to flat bed sowing, and the application of 30 or 60 kg P_2O_5/ha resulted in significantly higher grain yields compared to no phosphorus application. The combination of broad bed and furrows with phosphorus fertilization was found to be ideal for achieving higher productivity in greengram. The land configuration treatments had no impact on the productivity of wheat. The application of phosphorus to the preceding crop had a significant residual effect on the grain yield of wheat. The incorporation of greengram stover also significantly increased the grain yield of wheat. The increasing levels of N increased the grain yield of wheat significantly up to 80 kg/ha. The combination of greengram stover incorporation and 80 kg N/ha applied to wheat significantly increased the grain yield. Further, there was a significant interaction effect between the phosphorus applied to the preceding crop and N levels given to wheat on the grain yield of wheat.

Key words: greengram, wheat, cropping sequence, land configuration, residue management, nutrient economy

Introduction

The greengram (*Phaseolus radiatus* L.)–wheat (*Triticum aestivum* L.) cropping sequence is emerging as one of the major cropping systems for central and northern parts of India. Greengram is raised during the rainy season, i.e. *kharif* (July–October) with little or no irrigation support, followed by wheat in winter i.e. *rabi* (November–April) with a few life-saving irrigations. Being highly sensitive to water stagnation, greengram often suffers from-water logging following heavy rains (Ramachandrappa et al., 1990). Under such situations the applied nutrients lose effectiveness as well, resulting in poor nutrient use efficiency. This dissuades farmers from applying optimum fertilizer rates in greengram, fearing a low return per unit of fertilizer applied. Thus, the productivity of *kharif* greengram is very low at 379 kg/ha (GOI, 1997). In the

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succeeding *rabi* season wheat is also raised with limited water supplies and inadequate fertilizer rates. The prohibitive cost of fertilizer and the uncertainty of successful crop production and productivity are found to limit the use of appropriate fertilizer rates in wheat. Thus, a suitable water disposal system is necessary for greengram to overcome the adverse effect of water-logging. Besides promoting better nodulation and biological nitrogen fixation, this would also increase the efficiency of the fertilizers applied (Bhatia and Dastane, 1968). The incorporation of greengram residues after harvesting the pods of greengram improves the physico-chemical properties of the soil and reduces the mineral fertilizer requirement of the succeeding crop. Hence an attempt was made to study the effect of land configuration and phosphorus nutrition in *kharif*-grown greengram, and of crop residue management and nitrogen nutrition to the succeeding wheat crop, in order to find a suitable method of sowing and appropriate nutrient management practices for the greengram-wheat cropping sequence under limited water supply conditions.

Materials and methods

Site and soil

A two-year field experiment was carried out between 1997 and 1999 at the farm of the Indian Agricultural Research Institute, New Delhi (28° 38' N latitude,77° 11' E longitude and 228 m above sea level). The soil of the experimental site was alluvial sandy loam low in organic carbon and available phosphorus and medium in available potassium with a slightly alkaline reaction (Table 1). Rainfall was low to medium (264 mm and 186 mm spread over 12 and 14 days during the greengram and wheat growing periods, respectively) and medium (403 mm and 103 mm spread over 13 and 5 days for greengram and wheat growing, respectively) in the first and second years of experimentation.

Table 1
Physical and chemical properties of the experimental field

Particulars	'97–98	'98–99	Method followed
I. Mechanical analysis (%)			
Sand	63.4	64.1	
Silt	17.2	17.3	Bouyoucos hydrometer method (1)
Clay	19.4	18.6	
II. Chemical analysis			
Organic carbon (%)	0.41	0.40	Wet digestion method (2)
Available P_2O_5 (kg ha^{-1})	16.79	17.14	Olsen's method (3)
Available K_2O (kg ha^{-1})	169.82	172.43	Neutral normal ammonium acetate method (4)
pH (1:2.5)	7.23	7.41	Potentiometric method (4)
Electric conductivity at 25° C (dS m^{-1})	0.29	0.32	Solubridge method (5)

1: (Bouyoucos 1962); 2: (Walkley and Black,1934); 3: (Olsen et al., 1954); 4: (Jackson, 1967); 5: (Richards, 1954)

Field techniques

The greengram variety *Asha* (a tall, highly branching variety of 75–85 days duration with synchronous flowering) was sown in the second half of July with two methods of sowing: on a flat bed (FB) or a broad bed 2 m wide and furrows (BBF); and three levels of phosphorus: 0 (P_0), 30 (P_{30}) and 60 (P_{60}) kg P_2O_5/ha. The broad beds with furrows were formed by making furrows 30 cm deep and 30 cm wide on both sides of the 2 m wide bed. The sowing was done with a hand plough at a row spacing of 20 cm and a depth of 3–5 cm using a seed rate of 15 kg/ha in plots 36 m long and 2 m wide. The seeds were treated with appropriate strains of *Rhizobium* and phosphorus-solubilizing bacteria (*Pseudomonas striata*) culture before sowing. A uniform rate of 15 kg N/ha in the form of urea and phosphorus as per treatments in the form of single superphosphate (SSP) was applied by broadcasting before sowing and mixed into the soil. One hand weeding along with one intercultivation was done at 30 days after sowing (DAS) in greengram to control weeds. One spray of the systemic insecticide Rogar (dimethoate) was given to greengram during the pod formation stage. The mature pods of greengram were harvested by 2 manual pickings in the net plots (32 m long and 1.6 m wide). They were dried in the sun and threshed manually to separate the grains. The grains were sun-dried to a moisture content of 8% and the grain yield was noted after converting to t/ha. After the pod harvest, the greengram stover was either removed or incorporated *in situ* into the soil by disking and allowed to decompose for about three weeks before the sowing of wheat.

During the *rabi* season the wheat variety *Kundan* (a drought-tolerant, dwarf, high-yielding variety of 120–125 days duration) was sown in the third week of November at 5 cm depth using a tractor-mounted seed-drill with a row spacing of 20 cm and a seed rate of 100 kg/ha. The greengram plots were divided into eight sub-plots each 4 m long and 2 m wide to test combinations of two levels of residue management: greengram stover removed (SR) and stover incorporated (SI) and four levels of nitrogen: 0 (N_0), 40 (N_{40}), 80 (N_{80}) and 120 (N_{120}) kg N/ha. Half the nitrogen, in the form of urea, 30 kg P_2O_5/ha phosphorus in the form of SSP and 30 kg K_2O/ha potassium in the form of muriate of potash were applied by broadcasting and incorporated into the soil before sowing, while the remaining 50% nitrogen was given as top dressing about 30 DAS. One spray of isoproturon 25 DAS and one intercultivation was carried out to control weeds in wheat. Three irrigations, including one pre-sowing, were applied when moisture stress was the greatest during the crop growth period of wheat. The crop was harvested manually by cutting at ground level from a net plot 3.6 m long and 1.6 m wide, and threshed by mechanical thresher to separate the grains. The grain yield was recorded as t/ha.

Nodulation studies in greengram

At 40 DAS, five plants of greengram were selected at random in each plot and carefully uprooted without disturbing the entire root system and washed in tap water. The nodules were separated from the roots and their numbers were recorded. Afterwards the nodules were dried in a hot air oven and the dry weight was recorded. The average nodule number and nodule weight per plant were then calculated.

Chemical analysis

The available nitrogen, phosphorus and potassium status in the soil after the harvest of greengram and wheat, and the contents of these elements in the grain and stover of greengram and the grain and straw of wheat were estimated by standard procedures from the plant samples collected at the time of harvest (Jackson, 1967; Olsen et al., 1954). The total uptake of nitrogen, phosphorus and potassium was computed by multiplying their contents with the stover and grain yield of greengram and straw and the grain yield of wheat.

Statistical design and analysis

In greengram the experiment was laid out in a randomized block design (RBD) with three replications, while in wheat the treatments were laid out in a strip plot design (SPD) in 3 replications, with the *kharif* treatments to greengram in the horizontal strips and combinations of stover management and levels of nitrogen in the vertical strips. The analysis of data for both the crops was done as per the procedure given by Gomez and Gomez (1984) using a statistical programme written in Microsoft Excel 97 software. Differences between the treatments were estimated using the critical difference (CD) procedure where the F values were significant at the 0.05 probability level ($P < 0.05$). Regression equations were used to find the optimum N level for wheat for both years separately, as per the technique outlined by Boyd (1970), Neeteson and Waldman (1987) and Olness et al. (1998).

Results and discussion

Greengram

Nodulation

The nodule number and nodule weight recorded at 40 DAS were significantly higher in BBF as compared to FB. The application of both 30 and 60 kg P_2O_5/ha gave significantly higher nodule number and weight compared to no phosphorus application (Table 2). The interaction effect of land configuration and phosphorus levels was significant. Phosphorus levels combined with BBF produced significantly higher nodules per plant compared to FB. The reduced oxygen diffusion rate due to water-logging in flat bed sowing resulted in impaired nodulation in greengram (Singh et al., 1988). Thus, better nodulation was recored in BBF sowing, where water-logging was avoided. The combined complementary effect of BBF sowing and phosphorus application showed a significant improvement in nodulation in greengram.

Table 2
Nodulation pattern, grain yield and nutrient uptake of greengram as influenced by land configuration and phosphorus levels

Treatment	Nodules /plant		Nodule weight (mg/plant)		Grain yield (t/ha)		Nutrient uptake (kg/ha)					
							N		P		K	
	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Land configuration												
Flat bed	5.13	3.89	112.0	104.7	0.931	1.058	78.15	90.36	6.61	7.68	33.37	38.66
Broad bed and furrows	6.29	5.71	169.2	143.4	1.114	1.189	88.92	98.18	7.70	8.50	37.83	42.03
SEm±	0.13	0.18	3.5	4.2	0.016	0.011	1.12	0.78	0.10	0.08	0.50	0.41
LSD (P=0.05)	0.28	0.39	7.7	9.4	0.036	0.025	2.50	1.74	0.21	0.21	1.12	0.92
Levels of phosphorus (kg P_2O_5/ha)												
0	4.77	4.07	124.5	105.7	0.774	0.911	70.67	81.79	5.66	6.60	29.93	34.65
30	5.90	4.90	151.7	125.7	1.047	1.189	83.38	98.63	7.15	8.51	35.39	45.22
60	6.47	5.43	160.7	140.8	1.248	1.270	95.56	102.41	8.64	9.16	41.49	44.16
SEm±	0.15	0.21	4.3	5.2	0.020	0.014	1.38	0.96	0.12	0.10	0.62	0.50
LSD (P=0.05)	0.34	0.48	9.5	11.5	0.044	0.030	3.09	2.13	0.26	0.22	1.37	1.12

Grain yield

The grain yield was higher in BBF compared to FB sowing (Table 2). The increased grain yield may be attributed to better vegetative growth and nodulation and to the higher yield attributes observed in BBF compared to FB sowing. Similar observations of higher grain yield due to land configuration were reported in soybean (Tomar et al., 1996). The grain yield increased significantly with increasing levels of phosphorus up to 60 kg P_2O_5/ha. Phosphorus application increased the availability in the soil, resulting in improved growth and subsequent yield parameters, that finally translated into higher grain yield (Arya and Kalra, 1998). The response in grain yield due to phosphorus was linear in the first year ($Y=7.7900+0.1015x–0.0004x^2$) and quadratic in the second year ($Y=9.1100+0.1255x–0.0011x^2$). This may be attributed to the uneven and lower total rainfall in the first year, which limited phosphorus use efficiency, while the higher, more evenly distributed rainfall in the second year provided a favourable moisture status in the soil, enhancing phosphorus availability. The interaction effect of BBF and phosphorus levels was significant (Fig. 1). The application of 30 kg P_2O_5/ha in BBF produced higher grain yield than 60 kg P_2O_5/ha in FB sowing. This may be due to better root proliferation under BBF and the better use of phosphorus at both 30 and 60 kg P_2O_5/ha, while in FB the crop failed to make full use of applied phosphorus due to the restricted development of the roots and poor nodulation.

Nutrient content and uptake

The N, P and K content in the grains and stover were significantly higher in BBF sowing as compared to FB. The N content in both grain and stover increased up to 30 kg P_2O_5/ha compared with the control and was on par with 60 kg P_2O_5/ha. The P content in both grain and stover increased linearly from 0–60 kg P_2O_5/ha. The K content in the grains increased significantly at 30 kg P_2O_5/ha compared with the control (P_0) in the first year and at 60 kg P_2O_5/ha in the second year. The K content in the stover increased significantly with each level of phosphorus up to 60 kg P_2O_5/ha. The better soil conditions in BBF sowing and the increased availability of phosphorus after P application resulted in a higher content of N, P and K in both grain and stover. Likewise the total uptake of N, P and K, a function of nutrient content and dry matter production, was significantly higher in BBF compared to BF sowing (Table 2), mainly due to the higher total biomass production and higher contents of these nutrients. The higher uptake in BBF was on account of the improved drainage and better aeration. Increasing levels of phosphorus showed a significantly higher uptake of N, P and K. As phosphorus is essential for the better establishment of the root system and the functioning of root nodulation, it is obvious that it enhances the uptake of nitrogen. In the process, potassium uptake also increased, as some sort of proportion is maintained between N, P and K. The synergistic effect of phosphorus application on the uptake of N and P has also been reported (Wanothaman et al., 1991).

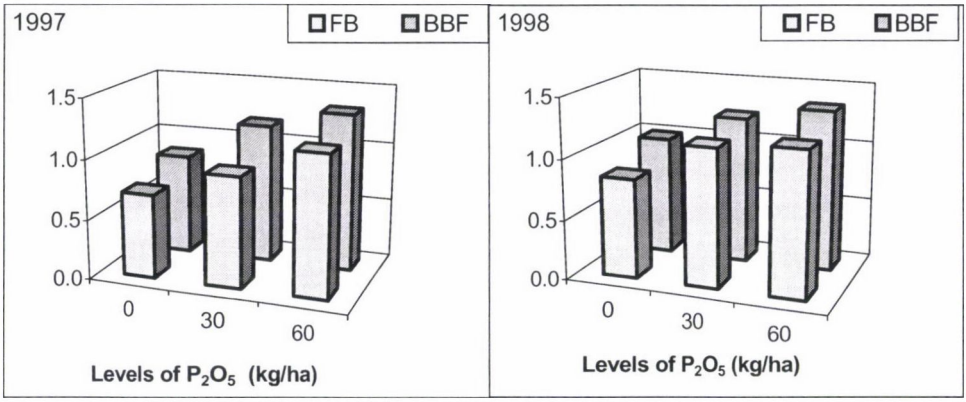

Fig. 1. Interaction effect of land configuration and phosphorus levels on the grain yield of greengram (FB: flat bed; BBF: broad bed and furrows)

Nutrient status in soil after greengram

The available N was higher in BBF as compared to FB sowing, while the available P was higher in FB as compared to BBF (Table 3). This may be due to the fact that the nodulation was better in BBF sowing, resulting in higher N_2 fixation and a carryover effect on the available nitrogen status in the soil. The phosphorus utilization was lower in FB as compared to BBF, resulting in higher available P status in the soil. The application of 30 and 60 kg P_2O_5/ha led to a significant increase in the available N and P in the soil after greengram. This may be due to the complementary effect of applied phosphorus on symbiotic activity, leading to higher biological nitrogen fixation and a consequent contribution to the available N pool in the soil. The levels of phosphorus applied also increased the available P status in the soil by virtue of being unutilized by the greengram. The available K status in the soil was unaffected by land configuration and phosphorus levels.

Wheat

Grain yield

The grain yield of wheat was not influenced by the land configuration treatments, implying that the advantage of BBF was reduced due to the limited water supply (Table 4). However, the application of 30–60 kg P_2O_5/ha to the preceding greengram crop resulted in a significantly higher grain yield over the control (P_0), indicating that there was a residual effect of phosphorus. The poorer utilization of phosphorus by greengram, and its subsequent availability to wheat resulted in higher vegetative growth and yield (Gill et al., 1987; Singh and Faroda, 1985). Greengram stover incorporation increased the grain yield of wheat significantly compared to treatments where the stover was removed. Besides influencing the physico-chemical properties of the soil, stover incorporation also increases nutrient availability and water conservation in the

soil. The increased grain yield of wheat due to the incorporation of previous crop residues was also reported by other workers (Tiwari et al., 1998). The grain yield of wheat only increased significantly with increasing levels of N up to 80 kg/ha. The increase in the grain yield due to the application of nitrogen has been amply demonstrated by earlier researchers (Mishra et al., 1994). The regression equations ($Y=42.1000+0.1875x-0.0009x^2$ in 1997–98 and $Y=41.6890+0.2455x-0.0011x^2$ in 1998–99) between the levels of nitrogen and the grain yield revealed that there was a quadratic relationship between the two, and the application of 91 kg N/ha in 1997–98 and 98 kg N/ha in 1998–99 was found to be optimum for obtaining a higher grain yield per unit of nitrogen applied.

There was a significant interaction effect of stover management and nitrogen levels on the grain yield of wheat (Fig. 2). The highest grain yield was recorded at 80 kg N/ha with stover incorporation. This was at par with the application of 120 kg N/ha with the stover removed, indicating a potential saving of 40 kg N/ha with stover incorporation. Similar reports on the additive effects of stover incorporation were observed earlier (Aggarwal et al., 1997; Tiwari et al., 1998).

There was a significant interaction effect between the phosphorus applied to the previous greengram crop and the nitrogen levels to wheat on the grain yield of wheat (Fig. 2). The application of 80 kg N/ha in plots receiving 30–60 kg P_2O_5/ha during the previous greengram season produced a wheat grain yield at par with the application of 120 kg N/ha, demonstrating the residual effect of phosphorus. This may be ascribed to balanced nutrition with phosphorus and nitrogen overcoming the bottleneck of the minimum law.

Nutrient content and uptake

The land configuration treatments, the levels of phosphorus to the preceding greengram crop and the stover management treatments had very little impact on the N, P and K contents in the grain and straw in either year, but the levels of nitrogen given to wheat resulted in a significant improvement in the N content in the grain and straw. The application of 80 and 120 kg N/ha resulted in a significantly higher N content. The P and K contents in the grain and straw were not influenced by the N levels. The land configuration treatments had no significant influence on the total uptake of N and P in wheat (Table 4). However, the total K uptake was significantly higher in BBF as compared to FB sowing. The application of 60 kg P_2O_5/ha to the preceding greengram crop led to significantly higher N, P and K uptake compared to no phosphorus, but this was at par with 30 kg P_2O_5/ha. Greengram stover incorporation also increased the total N, P and K uptake in wheat as compared to treatments where the stover was removed. There was a significant increase in the total uptake of N, P and K with every increasing level of nitrogen from 0–80 kg N/ha. A further increase to 120 kg N/ha gave only a slight increase and was found to be at par with 80 kg N/ha. This was mainly due to the increased availability at increasing levels of N. This is in conformity with the findings of earlier workers (Kumar et al., 1995).

Table 3
Available N, P_2O_5 and K_2O status (kg/ha) in the soil after the harvest of greengram as influenced by land configuration and phosphorus levels

Treatment	1997			1998		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Land configuration						
FB	279.19	17.17	175.90	281.21	17.26	176.34
BBF	285.68	17.11	176.34	284.34	17.21	176.79
SEm±	1.25	0.02	0.39	1.42	0.03	0.29
LSD (P=0.05)	2.78	0.05	NS	NS	NS	NS
Levels of phosphorus (kg P_2O_5/ha)						
0	273.50	16.99	175.50	275.06	17.07	176.19
30	283.97	17.12	176.04	284.31	17.29	176.56
60	289.84	17.32	176.83	288.95	17.34	176.93
SEm±	1.53	0.03	0.48	1.74	0.03	0.36
LSD (P=0.05)	3.40	0.07	NS	3.87	0.07	NS

FB: Flat bed; BF: Broad bed and furrows; NS: non-significant

Table 4
Grain yield and nutrient uptake of wheat as influenced by land configuration and levels of P to preceding greengram and by stover management and levels of N to wheat

Treatment	Grain yield (t/ha)		Nutrient uptake (kg/ha)					
			N		P		K	
	1997–98	1998–99	1997–98	1998–99	1997–98	1998–99	1997–98	1998–99
Land configuration								
FB	4.825	5.019	136.50	140.77	18.39	19.26	100.19	102.73
BBF	4.851	5.026	137.76	141.88	18.56	19.43	102.25	105.06
SEm±	0.018	0.025	0.69	0.82	0.08	0.12	0.67	0.91
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	1.48	2.04
Levels of phosphorus (kg P_2O_5/ha)								
0	4.728	4.962	134.08	139.35	18.02	19.02	99.29	101.36
30	4.847	5.026	137.34	141.34	18.52	19.39	101.33	104.67
60	4.939	5.081	139.69	143.29	18.89	19.62	103.04	105.65
SEm±	0.022	0.030	0.85	1.00	0.10	0.15	0.82	1.12
LSD (P=0.05)	0.048	0.067	1.89	2.23	0.22	0.33	1.82	2.49
Stover management								
SI	4.927	5.112	139.57	143.37	18.76	19.62	102.52	104.47
SR	4.750	4.933	134.51	139.38	18.20	19.07	99.91	103.32
SEm±	0.038	0.039	1.14	1.25	0.16	0.17	1.06	1.09
LSD (P=0.05)	0.080	0.085	2.45	2.29	0.35	0.36	2.28	NS
Levels of Nitrogen (kg N/ha)								
0	4.156	4.209	122.31	122.03	16.30	16.49	96.52	95.14
40	4.726	4.849	133.41	135.79	18.06	18.65	98.28	99.11
80	5.234	5.543	145.52	153.69	19.72	21.16	104.31	110.03
120	5.236	5.489	146.92	153.78	19.83	21.08	105.76	111.28
SEm±	0.053	0.056	1.61	1.77	0.23	0.24	1.50	1.55
LSD (P=0.05)	0.114	0.120	3.46	3.80	0.49	0.51	3.22	3.32

FB: Flat bed;; BBF: Broad bed and furrows; SI: Stover incorporation SR: Stover removed; NS: non-significant

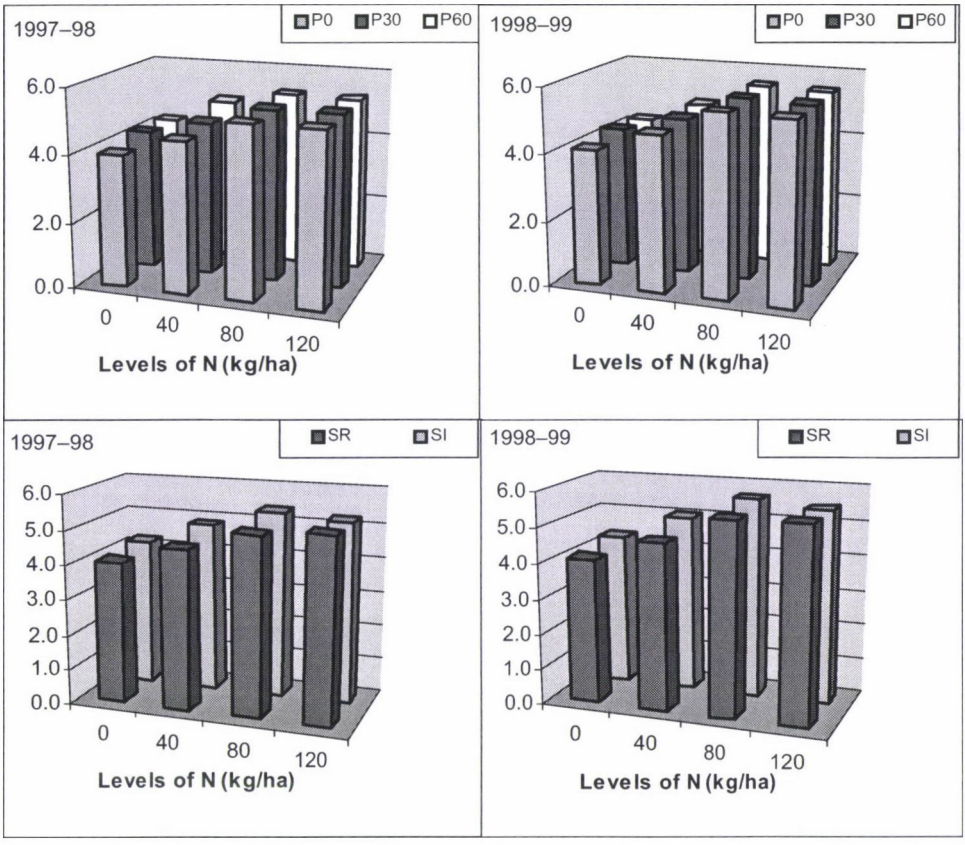

Fig. 2. Interaction effects of P levels to the preceding crop of greengram and N levels to wheat (upper diagrams) and the stover management and N levels to wheat (lower diagrams) on the grain yield of wheat; SR: stover removed, SI: stover incorporated

Nutrient status in soil after wheat

The available N, P and K status in the soil did not differ significantly after the harvest of wheat in any of the treatments. This may be due to the fact that wheat made full use of the variable nutrient availability found after greengram during its growth and neutralized this variation. The small differences observed were, however, statistically non-significant.

Conclusions

On the basis of the aforesaid results, it may be concluded that under conditions similar to those in the experiment the productivity of *kharif* greengram may be significantly enhanced by sowing the crop on broad beds 2 m wide with furrows, with the application of 30 or 60 kg P_2O_5/ha. Under such conditions, the optimum rate of phosphorus proved to be the application of 60 kg

P_2O_5/ha when soil moisture was limiting and 30 kg P_2O_5/ha when the rainfall was sufficient and evenly distributed. The succeeding crop of wheat in the *rabi* season benefited from the residual effect of the phosphorus applied to the preceding greengram crop. Greengram stover incorporation along with 80 kg N/ha to wheat led to a grain yield at par with that obtained when 120 kg N/ha was applied to wheat, indicating a saving of 40 kg N through greengram stover incorporation in a greengram-wheat cropping sequence under limited water supply conditions.

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EFFECTS OF DIFFERENT IRRIGATION REGIMES ON THE YIELD AND YIELD COMPONENTS OF DRY BEAN (*PHASEOLUS VULGARIS*)

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Water stress is one of the most important yield-limiting abiotic factors for dry beans (*Phaseolus vulgaris* L.). This study was conducted 1) to identify the effects of different irrigation scheduling on yield and yield components, 2) to define the number and intervals of irrigation water requirements in dry beans and 3) to compare the performances of two dry bean varieties in different irrigation schedules. The experiments were carried out in the fields of the Anatolian Agricultural Research Institute from 1992 to 1996. Two dry bean cultivars, Yunus90 and Karacasehir90, were used to study the effects of five irrigation schedules (S_1: High, S_2: Medium, S_3: Low, S_4: High-Low, S_5: Low-High rates of irrigation). The results indicated that year (Y) × irrigation regime (IR) interactions were important for yield and yield components. Karacaşehir90 was less affected by water stress than Yunus90 when rainfall was low in the growing season. Differences between irrigation schedules were more distinct when rainfall was low. The highest yield and yield component values were obtained from S_1, while the lowest values were obtained from S_3 and S_4. These results showed that water stress after flowering had the most adverse effect on yield. Thus, it is recommended that farmers use supplemental water chiefly after flowering when water sources are limited.

Key words: dry bean, *Phaseolus vulgaris,* irrigation, water stress

Introduction

Dry bean (*P. vulgaris* L.) has the greatest production of all the food legumes produced in the world (FAO, 2003). It is best grown in soils rich in organic matter and well drained, with or without irrigation depending on the amount of rainfall. Water stress is one of the major environmental factors limiting the bean yield, especially at the flowering stage (Walker and Hatfield, 1979).

The water content of plants ranges from 70 to 90% depending on the species, organs, development stages and environment (Willer and Donahue, 1990). There are critical stages in plant development when water stress or excess can cause significant decreases in yield. Many plant species do not have the capability of storing water to use in drought periods and need supplemental water during these periods. Thus, defining the appropriate time, amount and method of irrigation for each plant species will increase the efficiency of supplemental water. It is also important to know the root depth, the minimum water requirement at that depth and the water use efficiency of each species to increase irrigation benefits.

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Since bean plants have a shallow root system (Zaumeyer, 1954; Burman and Bohmont, 1961), they are more sensitive than deep-rooted plants to excessive amounts of water and show a better response to light irrigation at short intervals (Burman and Bohmont, 1961; Dubetz and Mahalle, 1969; Millar and Gardner, 1972; Robins and Domingo, 1956). The number of irrigations and the amount of water supplied on each occasion depend on the season, the soil type and depth, the organic matter in the soil and the tilt of the field surface (Mimms and Zaumeyer, 1947). Irrigation at 50% of available water was found to be the best treatment by Gungor (1981). Buckman and Brady (1960) also recommended irrigation at 50 to 85% of available water, with the warning that water stress might occur at 50%.

Excessive irrigation promotes vegetative growth and delays harvest as well as increasing root and shoot rots (Smittle, 1976). However, it is recommended to keep the soil surface moistened. Dry soil in the upper levels limits the uptake of water and nutrition by plants (Willer and Donahue, 1990). Other important factors affecting the irrigation amount and intervals are evapotranspiration losses and the water use efficiency of the plants. Water use efficiency depends on the variety, the yield potential of the variety and soil fertility (Allen et al., 2000).

Research has shown that the time of water stress is as important as the degree of this stress in beans (Maurer et al., 1969). Water stress between the early stages of flowering and seed setting causes significant yield decreases (Dubetz and Mahalle, 1969; Maurer and Goode, 1977; Willer and Donahue, 1990), sometimes up to 20% in beans (Smittle, 1976). Although preflowering irrigation is important to achieve maximum yield in bean (Buckman and Brady, 1960), opinions differ as to the importance of yield losses due to water stress at the vegetative stage (Şehirali, 1979). On the other hand, important yield losses were observed when there was water stress at the flowering and podding stages (Finn and Brun, 1980; MacKay and Eaves, 1962; Şehirali, 1979; Willer and Donahue, 1990). These losses were explained by a reduction in the number of flowers and pods formed (Dubetz and Mahalle, 1969; Smittle, 1976), and by blossom and pod drop (Borre, 1968).

The objectives of this study were 1) to identify how different irrigation regimes affect the yield and yield components of dry bean, 2) to find the most appropriate irrigation schedule for bean plants grown using supplemental water, and 3) to identify the responses of two dry bean cultivars to different irrigation regimes.

Materials and methods

This research was carried out in the fields of the Anatolian Agricultural Research Institute in Eskisehir for five years between 1992 and1996. The soil of the field is fine and silty with a pH of around 7.5. The field was prepared by ploughing at a depth of 15–20 cm in autumn, and 3 kg/da N and 6 kg/da P_2O_5 were applied preplanting in spring. Planting was done in the first two weeks of May.

A split plot design with four replications was used. The irrigation regimes were considered as the main plots and varieties were planted in subplots with 68 cm space between and 8–10 cm within rows. Each plot was planted with eight rows each 10 m long and a space of 3 m was left between the plots to avoid water escape from one plot to another. Furrow irrigation was applied and when the seedlings reached a height of 8–10 cm, irrigation channels were prepared.

Soil bulk density, field capacity and wilting point were calculated by taking soil samples from each 30 cm layer of the 0–90 cm soil profile in the field. Soil moisture was measured at 0–10, 10–20, 20–30 and 30–60 cm depth at preflowering to identify the time and amount of supplemental water given to each plot. Measurements at the 60–90 cm depth were added after flowering. A neutron moisture meter was used to measure soil moisture. Eighty neutron probe tubes, with two 150 cm long tubes in each plot, were used to measure soil moisture at two-day intervals. The amount of water given to each plot was controlled by a water gauge.

The two dry bean varieties used in the experiments have different plant types and seed sizes. Yunus90 is a Type I cultivar (determinate erect growth habit) with a 1000-seed weight of 455 g. Karacaşehir90 has a Type II growth habit (indeterminate with creeping growth) with a 1000-seed weight of 183 g.

The irrigation regimes (S_1 to S_5) were defined in relation to the amount of available water at certain points of the soil profile, where:

S_1: Irrigation to field capacity when the available water in the soil decreased to 75% of field capacity.

S_2: Irrigation to field capacity when the available water in the soil decreased to 50% of field capacity.

S_3: Irrigation to field capacity when the available water in the soil decreased to 25% of field capacity.

S_4: S_1 until flowering and S_3 after flowering.

S_5: S_3 until flowering and S_1 after flowering.

Observations were made on the emergence, flowering, podding and harvest dates as well as on diseases and weeds. Data on the number of pods/grains per plant and the number of grains per pod were calculated using 10 randomly chosen plants from each plot and the yield was calculated as kg/ha after the border rows were discarded.

The statistical analysis for all variables was done through ANOVA, and mean comparisons were made using Fisher's LSD at $P \leq 0.05$ (MSTATC).

Results

Although the experiments were conducted in five years, analysis of variance was performed for four years because data collection was not possible for most of the components in 1995. The results indicated significant differences between the irrigation regimes for yield, while this was not the case for the varieties (Table 1). Differences between the varieties were significant for pods per plant and grains per pod, and differences between the irrigation regimes were significant for pods per plant (Tables 2 and 3). Two two-way interactions (year × irrigation regime and year × varieties) were found for yield ($P < 0.01$) and two (year × varieties and irrigation regime × varieties) ($P < 0.01$ and $P < 0.05$, respectively) for pods per plant. The year × irrigation regime × varieties interaction was also significant for pods per plant ($P < 0.05$). Since the interactions were significant, the results are presented using interaction tables (Tables 4 and 5).

Table 1
ANOVA table for grain yields in the dry bean irrigation experiment

Source of variation	DF	SS	MS	F ratio	Prob>F
Year	3	488615.7	162871.9	185.358	<0.0001
Replication	12	18649.7	1554.1	1.769	0.0813
Irrigation Regime	4	230874.1	57718.5	65.687	<0.0001
Year × Irrigation Regime	12	33832.8	2819.4	3.209	0.0020
Error (A)	48	42177.1	878.7	–	–
Variety	1	1392.4	1392.4	3.270	0.0756
Year × Variety	3	26666.3	8888.8	20.872	<0.0001
Irrigation Regime × Variety	4	800.4	200.1	0.470	0.7576
Year × Irrigation Regime ×Variety	12	7727.3	643.9	1.512	0.1451
Error (B)	60	25551.8	425.9	–	–
Total	159	876287.4	–	–	–

Table 2
ANOVA table for pods per plant in the dry bean irrigation experiment

Source of variation	DF	SS	MS	F ratio	Prob>F
Year	3	3128.2	1042.7	108.053	<0.0001
Replication	12	575.2	47.9	4.967	<0.0001
Irrigation Regime	4	676.6	169.2	17.529	<0.0001
Year × Irrigation Regime	12	213.2	17.8	1.841	0.0678
Error (A)	48	463.2	9.7	1.086	0.3783
Variety	1	436.6	436.6	49.125	<0.0001
Year × Variety	3	146.5	48.8	5.495	0.0021
Irrigation Regime × Variety	4	89.9	22.5	2.530	0.0496
Year × Irrigation Regime ×Variety	12	236.1	19.7	2.214	0.0221
Error (B)	60	533.2	8.9	–	–
Total	159	6498.8	–	–	–

Table 3
ANOVA table for grains per pod in the dry bean irrigation experiment

Source of variation	DF	SS	MS	F ratio	Prob>F
Year	3	34.9	11.6	19.411	<0.0001
Replication	12	12.5	1.0	1.733	0.0890
Irrigation Regime	4	5.6	1.4	2.339	0.0685
Year × Irrigation Regime	12	7.0	0.6	0.968	0.4917
Error (A)	48	28.8	0.6	–	–
Variety	1	212.6	212.6	266.996	<0.0001
Year × Variety	3	5.6	1.9	2.328	0.0835
Irrigation Regime × Variety	4	1.5	0.4	0.468	0.7592
Year × Irrigation Regime ×Variety	12	12.3	1.0	1.288	0.2495
Error (B)	60	47.8	0.8	–	–
Total	159	368.6	–	–	–

Table 4
Effect of irrigation regimes on the grain yield, pods per plant and grains per pod of dry beans grown in Eskisehir

Irrigation regime	1992	1993	1994	1996	Mean
	Grain yield (kg/ha)				
S_1	21.7 *a**	37.8 *a*	32.7 *a*	34.1 *a*	31.6
S_2	18.5 *ab*	33.1 *bc*	30.4 *b*	32.0 *ab*	28.5
S_5	19.2 *a*	35.1 *ab*	27.6 *c*	29.7 *b*	27.9
S_4	15.2 *bc*	30.8 *cd*	25.2 *d*	22.3 *c*	23.4
S_3	13.8 *c*	27.9 *d*	23.8 *d*	18.4 *d*	21.0
LSD(0.05)	3.51	3.82	1.84	3.38	–
	Pods per plant				
S_1	30.8 *a*	16.5 *a*	19.3 *a*	18.3 *a*	21.2
S_5	28.1 *ab*	15.4 *ab*	17.3 *a*	15.2 *b*	19.0
S_2	22.7 *bc*	13.6 *b*	17.9 *a*	15.2 *b*	17.4
S_4	23.3 *bc*	13.4 *b*	15.0 *b*	13.7 *b*	16.3
S_3	22.0 *c*	14.3 *ab*	14.4 *b*	11.1 *c*	15.5
LSD(0.05)	5.6	2.4	2.2	2.0	–
	Grains per pod				
S_1	4.71 *a*	4.13 *a*	4.95 *a*	4.77 *a*	4.64
S_5	4.75 *a*	3.85 *a*	4.93 *a*	3.94 *b*	4.37
S_2	4.84 *a*	4.16 *a*	5.47 *a*	4.36 *ab*	4.71
S_4	4.55 *a*	4.27 *b*	5.45 *a*	3.95 *b*	4.56
S_3	4.05 *a*	3.55 *b*	5.27 *a*	3.91 *b*	4.20
LSD(0.05)	0.87	0.64	1.11	0.66	–

*Values followed by the same letter are not significantly different at the 5% level.

Table 5
Effect of varieties on the grain yield, pods per plant and grains per pod of dry beans grown in Eskişehir

Varieties	1992	1993	1994	1996	Mean
	Grain yield (kg/ha)				
Karacaşehir90	19.9 *a**	33.3 *a*	27.9 *a*	25.9 *b*	26.7
Yunus90	15.4 *b*	32.5 *a*	28.0 *a*	28.7 *a*	26.2
LSD (0.05)	1.46	1.81	1.08	1.08	–
	Pods per plant				
Karacaşehir90	27.9 *a*	17.0 *a*	14.8 *a*	18.4 *a*	19.5
Yunus90	22.9 *b*	12.3 *b*	14.6 *a*	15.1 *b*	16.2
LSD (0.05)	2.7	1.6	2.1	1.4	–
	Grains per pod				
Karacaşehir90	5.96 *a*	4.91 *a*	5.21 *a*	6.50 *a*	5.64
Yunus90	3.20 *b*	3.07 *b*	3.17 *b*	3.93 *b*	3.34
LSD (0.05)	0.33	0.47	0.91	0.53	–

*Values followed by the same letter are not significantly different at the 5% level.

The highest yields were obtained from S_1 in all years and the lowest from S_3 (Table 4). S_2 and S_5 were in the second group, while S_4 and S_3 were in the last group, according to their effect on the yield. Although the yield from S_1 was always higher when compared to S_2, the differences were only significant in two years (1993 and 1994). This was explained by the low precipitation and relative humidity values in these years (Table 6). The significance of the interaction could be due to the differences in rank observed between S_2 and S_5 in different years.

Table 5 shows the grain yields obtained from two bean varieties in four years. Karacaşehir90 yielded highest in 1992 and Yunus90 in 1996, while there was no significant yield difference between the two varieties in 1993 or 1994. The interaction between the irrigation regimes and the varieties was not important and both varieties responded to each irrigation regime similarly. The yield decreases due to water stress in Karacasehir90 and Yunus90 were similar (data not presented).

The number of pods per plant was affected by years, irrigation regimes and varieties (Table 2). The interactions of both years and irrigation regimes with varieties and also the three-way interaction were significant for pods per plant. The number of grains per pod was affected by the irrigation regimes at the 0.06 level, while the varieties had a significant effect on this component (Table 3). The effects of irrigation regimes and varieties on the number of pods per plant and grains per pod are presented in Tables 4 and 5, respectively. As can be seen in the tables, the irrigation regimes had a significant effect on the pods per plant in all years, but on the grains per pod in only two years (1993 and 1996). The highest pod number was found for Karacaşehir90 with the S_1 irrigation schedule in a high rainfall year, 1992. When rainfall was low at the flowering and podding stage in 1993, the number of pods significantly decreased and the lowest number of pods was obtained with the S_3 irrigation schedule in that year. The highest seed number was obtained from Karacaşehir90 at S_1, and the lowest seed number from Yunus90 with the S_3 irrigation regime.

Table 6
Climatic parameters during the growing seasons of dry beans in Eskişehir

Months	Climatic parameter	1992	1993	1994	1996
May	Precipitation (mm)	20.0	50.6	35.8	39.1
	Temperature (°C)	14.5	14.6	15.4	17.0
	Relative humidity	56	67	56	62
June	Precipitation (mm)	67.6	24.3	5.1	6.7
	Temperature (°C)	18.7	18.5	18.6	18.6
	Relative humidity	60	56	49	59
July	Precipitation (mm)	13.4	2.3	4.6	13.5
	Temperature (°C)	19.1	20.9	22.1	22.7
	Relative humidity	57	50	55	53
August	Precipitation (mm)	16.5	14.1	0.6	4.2
	Temperature (°C)	22.6	20.9	21.9	20.9
	Relative humidity	53	55	54	46

Discussion

Five irrigation regimes were found to have significant effects on the yield and yield components of dry beans. A high irrigation regime (S_1: irrigation to field capacity when available water decreased to 75% of field capacity) gave the highest yield in all years. This result was inconsistent with other irrigation response studies on beans (Maurer et al., 1969; Burman and Bohmont, 1961; MacKay and Eaves, 1962; Smittle, 1976; Miller and Burke, 1983) and peas (Maurer et al., 1968). The S_1 and S_2 irrigation regimes were both in the best performing group in high rainfall years, while S_1 out-yielded S_2 in low rainfall years. However, it is important to consider the number of irrigations that was required with each systems under Eskişehir conditions (Table 7). S_1 required irrigation on 12 occasions over a four-year average, while six irrigations were enough in S_2. Considering the 3.1 kg/ha yield difference between the two systems, the less frequent need for irrigation in S_2 has an advantage over S_1 in terms of economy and applicability. The frequency of irrigations in S_1 averaged 5.6 days, as opposed to 10.9 days in S_2 (data not presented). However, S_1 can be recommended if it is applied with a permanent irrigation system (such as dripper or sprinkler irrigation) under intensive, large field conditions, while S_2 is preferable for bean production fields on small family farms that are common in Eskişehir and on the Central Anatolian Plateau.

S_5 was in the second highest yielding group with S_2, but it required irrigation on 8 occasions during the growing season, which was more than S_2 (Table 7). This was expected, because the combination of precipitation decrease and temperature increase with the increasing water use of plants during the vegetative development stage later in the growing season increases evapotranspiration (Allen et al., 2000; Miller and Burke, 1983; Maurer et al., 1969) and requires frequent irrigation, since this regime shifted to S_1 application after flowering.

Table 7
Effect of irrigation regime on the number of irrigations required for dry beans in Eskişehir

Irrigation regime	1992	1993	1994	1996	Mean
	Number of irrigations				
S_1	10	11	15	13	12.3
S_2	6	5	7	6	6.0
S_3	3	3	3	2	2.8
S_4	6	7	7	4	6.0
S_5	8	7	11	6	8.0
Mean	6.6	6.6	8.6	6.2	

Water stress had a more dramatic effect on yield after the flowering stage than preflowering. The differences between S_4 and S_5 for yield and yield components were always significant and S_4 had low values, close to those recorded for S_3 (Table 4). Similar results were reported by Heatherly (1993) in soybean, Westgate (1994) in maize, and Robins and Domingo (1956), Maurer et al. (1969) and Kemp et al. (1974) in beans.

Water stress could be more effectively observed when rainfall was low (Table 6). There was little water stress at S_3 and S_4 when rainfall was high, although these two irrigation regimes caused severe water stress to bean plants when rainfall was low. Karacaşehir90 was less affected by water stress than Yunus90 in these years. This situation was related to the plant type of Karacaşehir90. The semi-dwarf (Type II) form of this variety enabled the plant canopy to cover the soil surface, thus reducing transpiration.

A comparison of the two varieties showed that Karacaşehir90 had a higher number of grains per pod than Yunus90. This character was less affected by the irrigation regimes than the pods per plant because of its high hereditary value. The yield differences observed between the irrigation regimes were largely due to differences in the number of pods per plant.

S_5 yielded more than S_4 in all years. This confirmed that dry beans are more sensitive to water stress after the flowering stage than preflowering. Similar results were reported by other researchers for beans (MacKay and Eaves, 1962; Robins and Domingo, 1956; Dubetz and Mahalle, 1969) and other crops (Heatherly, 1993; Westgate, 1994; Ney et al., 1994). Negative effects in the preflowering stage can be compensated for by adequate irrigation after flowering.

Based on the results of this research, a high level of irrigation (S_1) at every growth stage increased the dry bean yield. However, medium irrigation (S_2) may be more practical and economical if the yield obtained from the two systems is compared. If the water source is limited, farmers are recommended to use it chiefly after the flowering of bean plants.

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ORGANIC MANURES FOR INCREASED RICE PRODUCTIVITY AND SUSTAINED SUPPLY OF FE TO RICE

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Field experiments were carried out for 3 years to assess the efficacy of organic manures (*Sesbania, Leucaena,* cowpea, mungbean, wheat straw and FYM) in enhancing the productivity of rice and in supplying Fe. Green manuring with *Sesbania* gave the highest rice yield, whereas the lowest yield was recorded with wheat straw incorporation. The concentration and uptake of Fe by rice was significantly higher with organic manures. The Fe status of the soil after 3 crops of rice declined from the initial value, but the decline was least with FYM, followed by green manures. The application of organic manures is a good source for a sustained supply of Fe in soil.

Key words: organic manures, rice, Fe, *Sesbania, Leucaena,* cowpea, mungbean, wheat straw, FYM

Introduction

About 90% of world's rice is grown in Asia (IRRI, 1989), where it is the staple food and accounts for 50–80% of the calories consumed by the people of the region (Hossain and Pingali, 1998). To meet the expected rice consumption in the world in 2020, a compound growth rate of 1.2% is needed (Rosegrant et al., 1998). This growth rate has to be obtained by increasing productivity, as there is little scope for area expansion. The most widespread limitations to rice productivity are nutrient deficiencies, pest damage, soil constraints and water relations (Greenland, 1997). Among the micronutrients, deficiencies of zinc and iron are the most limiting factors for rice productivity. Fe deficiency is common in the calcareous soils that account for 30% of the global area (Takahashi et al., 2001).

India is a major producer of rice, accounting for 29.45% (44.5 million ha) of the global area, and rice is cultivated on a wide array of soils. The soils of the northwest plains are sandy, calcareous with high pH and highly permeable. In such soils Fe availability is limited due to excessive production of bicarbonates (HCO_3^-), which reduces Fe uptake by the crops (Rutland, 1971). Cropping systems involving high-yielding varieties of rice have contributed to an increase in micronutrient deficiencies in resource-poor areas due to enhanced harvests without adequate manuring. Fe deficiency is generally reported from dryland rice nurseries, upland rainfed rice fields, and rice fields having light sandy soils where water does not stand for a long time (Prasad, 1999). Submergence increases iron availability due to its reduction from Fe^{3+} to Fe^{2+}, so there is less Fe deficiency in such areas (Ponnamperuma, 1972).

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Iron malnutrition is now a global problem of immense proportions, as over 2 billion people, mostly women, infants and children, now suffer from a deficiency of Fe, I and/or vitamin A. Hence attempts have been made to enrich crops with micronutrients (Welch et al., 2002) and scientists have been successful in incorporating Provitamin A and iron into rice (Ahloowalia, 2000), which will go a long way to overcoming iron malnutrition in Asia, where rice is the staple food.

The Fe fertilization of calcareous soils results in the quick reversion of iron to less available forms, and therefore soil management through organic manures is advocated for correcting Fe deficiency (Nayyar and Takkar, 1989). By decreasing pH and Eh and by increasing the partial pressure of CO_2 (Sadana and Nayyar, 2000, Castilla and Salive, 2001) organic manures enhance the Fe concentration in the soil solution (Nayyar and Chhibba, 2000) and ameliorate Fe deficiency.

Integrated nutrient management (INM) through organic manures is not new to rice, and in recent years the use of cereal straws (Prasad et al. 1999a), dual-purpose legumes (John et al., 1989; Prasad et al., 1999b; Sharma and Prasad, 1999) and green manures (Duhan et al. 2002) have received considerable attention. However, most earlier studies focussed mainly on grain and straw yields and on nitrogen availability and uptake (Sharma et al., 1995; 2000; Aulakh et al., 2000) and very little information is available on the effect of INM on Fe concentration and uptake by rice. The present study was therefore carried out at the Indian Agricultural Research Institute (IARI), New Delhi to study the effect of INM on the concentration and uptake of Fe in rice.

Materials and methods

Site and soil

A field experiment was conducted at the IARI, New Delhi for 3 crop years (July–June 1992–93 to 1994–95). The experimental soil was a sandy clay loam Fluvent (52.8% sand, 21.5% silt, 25.7% clay) having pH 8.2 (1:2.5 soil to water ratio), 5800 kg ha^{-1} organic C, 558 kg ha^{-1} Kjeldahl N, 270 kg ha^{-1} alkaline permanganate-hydrolysable N, 22.6 kg ha^{-1} 0.5 M $NaHCO_3$ extractable P and 177 kg ha^{-1} 1 N NH_4OAC exchangeable K, as determined using the procedures described by Prasad (1998). The available (DTPA-extractable) Fe in soil, as determined using the procedure described by Singh et al. (1999), was 6.1 mg kg^{-1}.

Experimental design and treatments

The experiment was conducted in a randomized block design having three replications. The seven treatments were *Sesbania aculeata,* cowpea (*Vigna unguiculata*) green manuring, *Leucaena leucocephala* green leaf manuring, mungbean (*Vigna radiata*) green manuring after pod picking, chopped wheat straw incorporation, farmyard manure (FYM) and a control.

Field techniques

Sesbania and cowpea green manures were sown in the third week of April and received 20 kg ha^{-1} N as urea and 17 kg ha^{-1} P as ordinary superphosphate. They were incorporated into the soil in the first week of July, 2–3 days before transplanting the rice. *Leucaena* loppings (2 t/ha/yr),

wheat straw (5 t/ha/yr) and FYM (10 t/ha/yr) were incorporated into the soil 1 month before transplanting rice (first week of June) for their proper decomposition. The summer mungbean variety Pusa 16 was sown in the last week of April and the residue was incorporated as green manure after picking pods in the last week of June. The control plot was left as summer fallow. The amount of nutrients added/recycled by these treatments is given in Table 1.

Rice (variety P-615) was transplanted in the first week of July after the plots were bunded, flooded with water and puddled. Rice received 20 kg ha^{-1} P as ordinary super-phosphate and 30 kg ha^{-1} K as muriate of potash. Two to three 25–30-day-old seedlings were transplanted/hill at 25 cm × 10 cm spacing. All the P and K were applied during land preparation, while nitrogen (80 kg/ha) was applied in 2 split doses, the first half-dose 10 days after transplanting and the rest at panicle initiation. The rice was harvested in the first week of November.

Chemical analysis

The Fe concentration in the rice grain and straw and the DTPA-extractable available Fe in the soil was determined using the procedure described by Singh et al. (1999) on an atomic absorption spectrophotometer. The Fe concentration was multiplied by the respective grain/straw yield to obtain the uptake by grain/straw and these figures were added to obtain the total uptake of Fe by the rice crop.

Table 1

Quantities (t $ha^{-1}yr^{-1}$) of organic residues added and their nutrient contents (mg/kg dry matter)

Organic manure	Quantity applied	Nutrient concentration				Fe addition (g/ha)
		N (%)	P (%)	K (%)	Fe (mg/kg)	
FYM	10.0	0.42	0.25	0.51	45	450
Wheat straw	5.0	0.40	0.10	1.10	10	50
Sesbania GM	5.5	2.60	0.40	2.20	140	770
Cowpea GM	3.5	1.70	0.45	0.72	90	315
Leucaena GM	2.0	2.42	0.27	1.40	100	200
Mungbean GM	1.9	1.52	0.21	0.74	235	447

*GM: Green Manuring

Results and discussion

Grain yield

The grain and straw yield of rice was significantly influenced by organic manures (Table 2). The incorporation of wheat straw and FYM had no significant impact on the rice grain yield, while *Sesbania* and cowpea green manuring led to significantly more grain than *Leucaena* green leaf manuring, which in turn produced more grain than the control, FYM and wheat straw treatments. This was primarily due to the additional supply of NPK, especially N, which was the highest in the case of *Sesbania* and cowpea green manuring. The grain and straw yield of rice as a whole was significantly increased by the application of organic manures, compared with the moderate level of N applied to rice in the fallow (check) plots. The mean increase in rice grain yield due to organic manures compared with the fallow plots ranged from 1.8% in FYM plots to 15.3% in *Sesbania* green manured plots.

Table 2
Influence of organic manure on grain and straw yield (t/ha) of rice (average of 3 years)

Treatment	Rice yield (t/ha)	
	Grain	Straw
Sesbania GM	5.0	8.9
Cowpea GM	5.0	8.8
Leucaena GM	4.7	8.5
Mungbean GM	4.5	8.4
Wheat straw	4.2	7.8
FYM	4.4	8.7
Fallow	4.4	7.5
LSD (P=0.05)	0.3	0.2

Aulakh et al. (2000) also demonstrated that with moderate N fertilization, green manuring with *Sesbania* gave a higher yield of rice. Similarly, Sharma and Prasad (1999) and Prasad et al. (1999b) showed that summer mungbean could lead to a nitrogen economy of 98–113 kg/ha in a rice-wheat cropping system. There was a decline in the rice yield of plots receiving wheat straw, as compared to fallow, due to the immobilization of native N due to the high C: N ratio (Mary et al., 1996). Yadav et al. (2000) reported that the rice grain yield in treatments receiving 50% of the recommended N dose through wheat residues was in general significantly lower by 4–18% than the yield obtained with the recommended fertilizer NPK dose.

Fe concentration

The concentration of Fe in rice grain and straw was the least in fallow plots (no organic manures) and was significantly lower than in all the organic manure treatments (Table 3). Of the organic manures, FYM supplied the largest amount of Fe to the soil (Table 1) and gave the highest concentration of Fe in rice grain and straw. Wheat straw and *Leucaena* leaf manure generally gave the lowest concentration of Fe in rice grain and straw. The concentration of iron in rice grain was less than that in straw. On average, the Fe concentration was 1.68 times higher in the straw than in the grain.

Fe uptake

The Fe uptake by rice (grain, straw and total) was the highest in plots green-manured with *Sesbania,* cowpea, *Leucaena* and mungbean and was significantly higher than in the FYM treatment, which in turn was superior to the wheat straw and fallow plots (Table 3). Of the total Fe taken up by rice, about 23% was retained in the grain and the rest in the straw. The high Fe concentration in the straw (1.68 times that in the grain) and the higher straw yield (2.08 times that of grain) in rice resulted in higher uptake of Fe by rice straw.

Table 3
Fe concentration (mg/kg) and uptake (kg/ha) by rice and DTPA-extractable Fe in the soil after three crops of rice, as influenced by the addition of organic manures (average of 3 years)

Treatments	Fe concentration		Fe uptake			Fe content of soil (mg/kg)
	Grain	Straw	Grain	Straw	Total	
Sesbania GM	291.6	475.3	1.48	4.87	6.35	5.89
Cowpea GM	293.3	473.1	1.43	4.89	6.32	5.86
Leucaena GM	285.7	470.3	1.36	4.66	6.14	5.82
Mungbean GM	291.4	474.7	1.42	4.66	6.08	5.85
Wheat straw	286.6	465.9	1.28	4.16	5.45	5.85
FYM	295.9	491.1	1.34	4.52	5.86	5.94
Fallow	275.7	457.3	1.17	4.13	5.30	5.80
LSD (P=0.05)	10.5	13.5	0.13	0.29	0.40	0.04

Initial Fe status: 6.1 (mg/kg)

Large rice harvests needing heavy inputs of major plant nutrients have made farmers shift to inorganic fertilizers, so now hardly any organic manures are applied. Even rice straw is being burned *in situ* to overcome the short turnover time in cropping systems such as rice-wheat, and a large amount of plant nutrients which could be recycled are lost. Furthermore, increased income has permitted the rice farmers to buy tractors and the increased mechanization has gradually eliminated the draught animals from the farms, resulting in the reduced availability of farmyard manure. All this has resulted in a widespread deficiency of micronutrients (Takkar, 1996). The present study shows that a substantial proportion of the Fe needs of rice can be met through green manuring with *Sesbania,* cowpea or dual-purpose legumes such as mungbean.

Available Fe status of soil after three cycles of the system

The Fe status of the soil declined in general from the initial levels after the rice crops, but organic manures prevented this decline. The Fe content of the soil after three cycles of rice was the highest with FYM, followed by *Sesbania* and cowpea green manuring, which in turn were followed by wheat straw incorporation. *Leucaena* green leaf manuring was also better than the control plots, which had the lowest DTPA-extractable Fe in the soil. The fallow plots had the lowest soil Fe content, but this was at par with plots treated with *Leucaena* leaf manure.

The present study shows that green manures and FYM can provide a sustained supply of Fe to rice and also help in maintaining higher available Fe. Wheat straw incorporation did not increase the Fe uptake by rice, but maintained a significantly higher DTPA-extractable Fe content in the soil after three rice crops. Integrated nutrient management involving the use of organic manure with chemical fertilizers is strongly recommended for sustained rice production.

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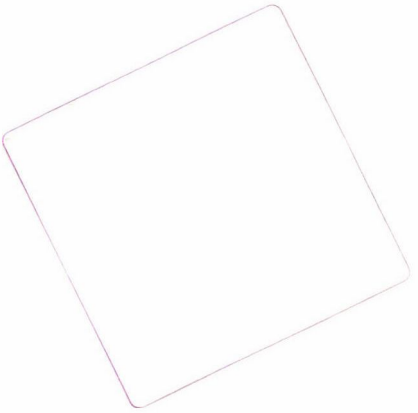

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EFFECT OF SRI (SYSTEM OF RICE INTENSIFICATION) PRACTICES ON THE YIELD ATTRIBUTES, YIELD AND WATER PRODUCTIVITY OF RICE (*ORYZA SATIVA* L.)

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Field experiments were conducted during the wet and dry seasons of 2002 and 2003 at Tamil Nadu Agricultural University Farm, Coimbatore, India to study the effect of practices recommended in the System of Rice Intensification (SRI) on the yield attributes, yield and water productivity of rice (*Oryza sativa* L.). The experiments were laid out in a randomized block design with three replications. The treatments were i) using 21-day-old (conventional) or 14-day-old (dapog nursery) seedlings; ii) crop geometry at 15 × 10 cm, 20 × 20 cm or 25 × 25 cm; iii) irrigation at 5.0 cm depth (conventional) or 2.0 cm depth when hair-line cracks developed (SRI); iv) weed control (conventional and SRI weeding), and v) nitrogen management (recommended and LCC-based N application) during the wet season of 2002. During the second crop season (dry season, 2003), all the treatments were repeated except nitrogen management, since there was no response to LCC-based N in the wet season. The treatments were slightly modified based on the results of the wet season crop. The yield attributes (panicle length, number of panicles hill^{-1}, total number of grains panicle^{-1}) were significantly higher in the treatment involving 14-day-old seedlings + 25 × 25 cm spacing + water-saving irrigation + LCC-based N management + SRI weeding than in the other treatments during the wet season. During the dry season, greater values of panicle length, no. of panicles hill^{-1} and filled grains panicle^{-1} were recorded in the treatment combination involving 14-day-old seedlings + 25 × 25 cm spacing + water-saving irrigation + SRI weeding. The grain yield and water productivity were significantly increased when applying SRI weeding with 14-day-old dapog seedlings planted at 25 × 25 cm spacing to achieve yields of 7009 and 5655 kg ha^{-1}, and 0.610 kg and 0.494 kg per m^3 of water , respectively, in the wet and dry seasons.

Key words: rice, yield attributes, yield, water productivity, system of rice intensification

Introduction

The total yield of rice, the 'Global Grain' grown in 89 nations, is almost 518 million tonnes every year and it is the staple food for more than half of the global population. In India, it occupies about 44.6 million hectares with a production of 86.0 million tonnes and it continues to hold the key to sustainable food production by contributing 20–25% of agriculture GDP and assuring food security in India for more than half of the total population (Anonymous, 2002). Exports of rice steadily increased from 0.4 million tonnes in the mid-eighties to 5.0 million tonnes by 1995–96 and earned Rs. 30 billion (~700 million US $) through foreign exchange (Thiyagarajan and Selvaraju, 2001). The burgeoning

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population of India is expected to stabilize at around 1.4 billion by 2025 and 1.6 billion by 2050, requiring an annual 380 and 450 million tonnes of food grains, respectively (Siddiq, 2000), but many challenges must be faced in the quest to overcome food scarcity with the limited resources available for agriculture. Though India tops the list in terms of area, having 28% of the world's rice-growing area (Ram and Vyas, 1997), the productivity is very low compared to leading rice-growing countries. The major constraint in rice production is the lack of suitable crop management practices and sufficient irrigation facilities. The recommended water management practice for rice is to provide irrigation up to 5 cm depth, one day after the disappearance of ponded water (TNAU, 1999). However, many farmers keep their fields under flooded conditions during the entire growing period, which is a wasteful practice. The total water input in rice fields varies widely between 500 and 3000 mm depending on the environmental conditions and the length of the growing period (Bouman and Tuong, 2000). Considering the future food requirements, competition from non-agricultural uses for fresh water, and the large amount of water currently used in rice cropping, new methods of rice cultivation must be identified, aiming at lower water requirements and higher crop productivity. Earlier studies revealed that rice can grow very well under semi-aquatic conditions with little or no major reduction in yield, and it has a self-adjusting nature which will have a synergetic effect on rice growth and yield. This has been derived from the concept of the System of Rice Intensification (SRI) developed by Fr. Henri de Laulanie along with farmers in Madagascar (Uphoff, 1999). This system is composed of a package of agronomic measures that should be applied simultaneously to achieve a yield increase. Uphoff (2001) reported that the components of SRI include the transplanting of young seedlings, usually 8–12 days and not more than 15 days old (4^{th} phyllochron). This preserves a potential for tillering and rooting that is reduced if transplanting occurs after the 4^{th} phyllochron. Seedlings are transplanted singly and very carefully to cause minimum trauma to the young plants. Transplanting the seedlings with wider spacing in a square pattern facilitates better weeding operations using a mechanical weeder and consequent aeration of the soil. This gives more room for better root and canopy growth. The soil is kept moist but not inundated during the vegetative growth phase, so that the soil is aerated and never becomes hypoxic. Early and frequent weeding is essential, because otherwise weed growth will become a problem. Weeding should be started about 10 days after transplanting using a rotary hoe that churns up the surface soil to remove weeds and provides additional soil aeration. The practice of SRI is not only aimed at maximum yield but at promoting the higher productivity of land, labour, capital and water in ways that benefit the farmer, especially poor ones. Many countries like Indonesia, Madagascar, Bangladesh, etc. reported a double or threefold increase in the rice grain yield with less water consumption. It was against this background that the field investigation was carried out to study the effect of SRI practices on the yield attributes, yield and water productivity of rice (*Oryza sativa* L.).

Materials and methods

Field experiments on rice were carried out at Tamil Nadu Agricultural University, Coimbatore, India during the wet and dry seasons of 2002 and 2003. Coimbatore is situated in the Northwestern agro-climatic zone of Tamil Nadu at 11°N latitude and 77°E longitude and at an altitude of 426.7 m above mean sea level. The soil of the experimental field was a deep, moderately well-drained clay loam, low in available N (244 kg N ha^{-1}), medium in available P (17.2 kg P_2O_5 ha^{-1}) and high in available K (560 kg K_2O ha^{-1}). The electrical conductivity of the soil was 0.80 dS m^{-1} and the pH was 8.2. Mechanical analysis of the soil showed 18.2 %, 18.1%, 19.0% and 44.2% of coarse sand, fine sand, silt and clay, respectively. The rice variety CO.47, with a field duration of 110 days, was used in the trial. The dates of sowing and harvest were 11 Jul. and 7 Nov. in 2002 and 23 Jan. and 21 May in 2003, respectively.

Based on the previous year's findings for individual technologies, the main objective was to study only the combined effect of these individual technologies as a package under SRI practices. The experiments were laid out in a randomized block design replicated thrice. The selected packages of treatment details are presented in Tables 1 and 2.

Table 1
Treatment details (Experiment I –Wet season – 2002)

Treatment	Seedling age (days)	Spacing (cm)	Irrigation	Nitrogen	Weeding
T_1	21	15 × 10	Conventional	Recommended	Conventional
T_2	21	15 × 10	Conventional	LCC	Conventional
T_3	21	15 × 10	Water-saving	Recommended	Conventional
T_4	21	15 × 10	Water-saving	LCC	Conventional
T_5	14	20 × 20	Conventional	Recommended	Conventional
T_6	14	20 × 20	Water-saving	Recommended	Conventional
T_7	14	20 × 20	Water-saving	LCC	Conventional
T_8	14	20 × 20	Water-saving	Recommended	SRI Weeding
T_9	14	20 × 20	Water-saving	LCC	SRI Weeding
T_{10}	14	25 × 25	Conventional	Recommended	Conventional
T_{11}	14	25 × 25	Water-saving	Recommended	Conventional
T_{12}	14	25 × 25	Water-saving	LCC	Conventional
T_{13}	14	25 × 25	Water-saving	Recommended	SRI Weeding
T_{14}	14	25 × 25	Water-saving	LCC	SRI Weeding

LCC = N management based on the Leaf Colour Chart

The result of Experiment I revealed that younger seedlings (14 days old) from a dapog nursery gave higher yields than conventional seedlings. In the dapog nursery seedlings are raised on a surface such as a banana leaf so that they can be easily transported to the field and transplanted at a young age. The nitrogen requirement based on the Leaf Colour Chart (LCC) was 140 kg N ha^{-1}, whereas it was only 120 kg N ha^{-1} in recommended practice. However, there was no significant difference in yield between LCC and traditional fertilization. The cost of fertilization was also higher in LCC than traditional fertilization. So it was decided to include both younger seedlings (14 days old) from a dapog nursery and aged seedlings (21 days) from a conventional nursery, and to exclude the LCC treatment and retain recommended N management for all the treatments in Experiment II (dry season). The modified treatment details of Experiment II are furnished below.

Table 2
Treatment details (Experiment II – dry season – 2003)

Treatment	Seedling age (days)	Spacing (cm)	Irrigation	Weeding
T_1	21	15 × 10	Conventional	Conventional
T_2	21	15 × 10	Water-saving	Conventional
T_3	14	15 × 10	Conventional	Conventional
T_4	14	15 × 10	Water-saving	Conventional
T_5	21	20 × 20	Conventional	Conventional
T_6	21	20 × 20	Conventional	SRI weeding
T_7	21	20 × 20	Water-saving	SRI weeding
T_8	14	20 × 20	Conventional	Conventional
T_9	14	20 × 20	Conventional	SRI weeding
T_{10}	14	20 × 20	Water-saving	SRI weeding
T_{11}	21	25 × 25	Conventional	Conventional
T_{12}	21	25 × 25	Conventional	SRI weeding
T_{13}	21	25 × 25	Water-saving	SRI weeding
T_{14}	14	25 × 25	Conventional	Conventional
T_{15}	14	25 × 25	Conventional	SRI weeding
T_{16}	14	25 × 25	Water-saving	SRI weeding

The recommended rate of N, P and K (120: 38: 38 kg ha^{-1}) was applied as urea (46% N), single superphosphate (16% P_2O_5) and muriate of potash (60% K_2O), respectively. Nitrogen was applied either at the recommended level or according to the LCC schedule depending upon the treatment. In the recommended practice, N was applied in four splits: 1/6 at 7 days after transplanting (DAT), 1/3 at 21 DAT, 1/3 at panicle initiation (PI), 1/6 at first flowering (FF). In LCC-based nitrogen management treatment, the LCC values were recorded as per the standard procedure (IRRI, 1996) at weekly intervals starting from 14 DAT to flowering. Whenever the LCC values were found to be below the fixed critical level (No. 4), 35 kg of N ha^{-1} was applied. The full dose of P_2O_5 was applied basally to all the treatments. Potassium was applied in four splits: 25% at 7 DAT and 25% each at the active tillering (AT), PI and FF stages. During both the seasons 25 kg of zinc sulphate ha^{-1} was applied to the crop as basal dressing.

In conventional irrigation (CI) the crop was irrigated to a depth of 5 cm one day after the disappearance of ponded water from planting to maturity. Irrigation was stopped 10 days prior to harvest. In water-saving irrigation (WSI) a 2 cm depth of water was given throughout the crop growth period on the development of hairline cracks. Irrigation was stopped 10 days prior to harvest.

In conventional weeding the emulsifiable concentrate of butachlor 1.25 kg ha^{-1} was applied as a pre-emergence herbicide mixed with sand (50 kg ha^{-1}) at 3 DAT with about 2 cm of standing water, and hand weeding was done twice (20 and 45 DAT). In SRI weeding a hand-operated rotary weeder was used to incorporate weeds with simultaneous stirring up of the soil. The weeder was operated between the rows in both directions. A total of five mechanical weedings were given to the SRI weeding plots at 7-day intervals during both the wet and dry seasons from 12 days after transplanting.

The amount of rainfall received during the cropping period was 356 mm and 215 mm distributed over 22 and 13 rainy days in the wet and dry seasons, respectively. The amount of irrigation water per plot was recorded and computed using the irrigation measuring device 'Parshall flume' and expressed as litres per plot and mm ha^{-1} per irrigation. A mark was made at five places per plot either at 2 cm or 5 cm, depending on the treatment, and the water was allowed up to the level of the mark, after which the quantity of water was calculated. The water productivity was calculated for each irrigation treatment and expressed in kg m^{-3}. The following formula was used to calculate water productivity:

$$\text{Water productivity} = \frac{\text{Grain yield (kg/ha)}}{\text{Total water consumed in } m^3\text{/ha including effective rainfall}}$$

The yield attributes recorded in the study were panicle length, panicle weight, number of panicles $hill^{-1}$, thousand grain weight, total number of seeds $panicle^{-1}$, number of filled seeds $panicle^{-1}$, sterility percentage and number of productive tillers m^{-2}. One week before harvest 100 (15 × 10 cm) and 75 hills per plot (20 × 20 cm, 25 × 25 cm) were counted from the third row on each side (consecutively) or randomly from the net plot to calculate the total number of panicles $hill^{-1}$. Panicles from five hills were taken prior to harvest and the panicle length was recorded from the point of ligules to the tip of the panicle and expressed in cm. The number of panicles was counted for five sampled hills and single panicle weight was calculated from the total panicle weight and expressed in g. The panicles from the five sampled plants at harvest were separated and dried in an oven at 70°C for 24 hours. After 24 hours all the spikelets were removed from the panicles and filled and unfilled grains were separated. One thousand grains were counted from filled grains and 1000 chaffs from the unfilled grains to derive the single grain and chaff weights. The total number of filled grains and unfilled chaffs was caéciéated. The number of filled and unfilled grains $panicle^{-1}$ in the sampled panicles were counted and the sterility percentage was calculated as the total number of grains per panicle to the number of unfilled grains per panicle. One thousand filled grains were taken from each plot and their weight was recorded at 14% moisture content and expressed in g. The seed yield from each net plot area was recorded at 14% moisture level and expressed in kg ha^{-1}. The data collected were analysed statistically following the procedure given by Gomez and Gomez (1984). Wherever the treatment differences were significant, critical differences were calculated at the 5% probability level.

Results and discussion

Yield attributes

Most of the yield components were significantly improved by a combination of younger seedlings transplanted before the 4th phyllochron (14 days old), wider spacing with a plant density of 16 seedlings m^{-2} (25 × 25 cm), either conventional irrigation or limited irrigation and mechanical weeding. In this combination, increased leaf area and a subsequent increase in photosynthetic activity were exhibited through increased biomass production, as a major portion of the photosynthates accounted for dry matter and all these factors favoured the yield components under SRI practices (Senthilkumar, 2002). Wider spacing was the reason for less below- and aboveground competition, giving better grain filling, higher grain weight and a larger number of filled grains per panicle (Rajesh and Thanunathan, 2003). An optimum supply of irrigation water with mechanical weeding led to higher nutrient availability, subsequently resulting in better source to sink conversion and in turn enhancing the production of a higher total number of seeds and filled seeds $panicle^{-1}$. The above discussion on the yield characters of rice points out that the number of filled seeds, the total number of grains and the test weight were influenced by agronomic manipulations like younger seedlings, limited irrigation, wider spacing and mechanical weeding practices (Tables 3 and 4).

Grain yield

The grain yield of rice was significantly influenced by the various SRI management factors tested in both the years. The combination of younger seedlings (14 days old), wider spacing (25 × 25 cm), limited irrigation with 2 cm water at the hairline crack development stage, with the incorporation of weeds

using a mechanical weeder gave the highest grain yield. Alwar Arunachalam (1984) reported that younger seedlings from a dapog nursery increased the dry matter production at all the growth stages and produced higher grain and straw yields compared to 30-day-old seedlings from conventional nurseries or double transplanting nurseries. Transplanting seedlings at a younger stage provides sufficient nutrients for vegetative growth and also for the reproductive phase, which ultimately leads to increased plant height and yield attributes, thereby resulting in increased grain and straw yields (Krishna, 2000).

Almost all the yield-attributing traits except sterility percentage and productive tillers m^{-2} were favourably influenced by a wider spacing of 25 × 25 cm. This might be due to the efficient utilization of resources and to less inter- and intra-space competition between widely-spaced plants, resulting in superior yield attributes and consequently increased yield. These results are in accordance with the findings of Padmavati et al. (1998). A decrease in leaf area causes a reduction in the area available for the interception and absorption of the specific wavelength of light necessary for photosynthesis. Greater photosynthetic area, leaf area index and TDMP (total dry matter production) after panicle initiation contribute to increased yield attributes and consequently increased grain and straw yields. Similarly, wider spacing led to higher grain yield than narrow spacing due to the fact that more space and nutrients were available to individual plants (Shad, 1986). Wider spacing did not affect the grain and straw yields or the harvest index in the work of Jagannath et al. (1998) and Shrirame et al. (2000).

Table 3

Effect of system of rice intensification (SRI) practices on yield attributes, yield and water productivity of rice in the wet season (2002)

1	2	3	4	5	6	7	8	9	10	11
T_1	18.13	1.780	6.556	18.45	98.58	81.23	17.83	437	5651	0.3214
T_2	18.45	1.801	6.586	18.24	106.95	84.11	20.52	439	5696	0.3239
T_3	18.12	1.790	6.436	17.99	108.85	83.75	23.75	429	5600	0.4298
T_4	18.02	1.762	6.421	17.85	109.84	81.81	26.26	427	5490	0.4214
T_5	20.11	2.001	16.120	19.14	121.42	87.64	28.17	403	6289	0.3919
T_6	20.48	1.826	16.120	18.61	133.51	85.56	36.06	403	5997	0.5217
T_7	19.71	1.829	16.400	18.56	125.44	86.45	31.03	410	6062	0.5274
T_8	20.54	2.017	16.640	18.67	127.53	89.38	29.94	416	6577	0.5722
T_9	20.57	1.998	16.800	18.49	135.49	89.29	31.65	420	6603	0.5744
T_{10}	20.73	2.025	24.875	18.74	145.51	95.44	29.74	398	6325	0.3941
T_{11}	20.75	2.019	25.063	18.72	144.33	92.62	36.13	401	6308	0.5488
T_{12}	19.25	1.966	24.500	19.1	130.49	93.54	31.72	392	6283	0.5466
T_{13}	20.89	2.111	25.688	19.73	137.47	99.41	27.71	411	7009	0.6097
T_{14}	20.96	2.004	25.875	19.01	145.53	92.47	37.23	414	6919	0.6019
SEd	0.21	0.054	1.582	0.36	3.94	3.38	1.56	42	214	0.0176
$CD_{P=0.05}$	0.43	0.111	3.252	0.74	8.10	6.95	3.21	NS	439	0.0359

1: Treatment; 2: Panicle length (cm); 3: Panicle weight (g); 4: No. of panicles/hill; 5: 1000 grain weight (g); 6: Total No. of grains/panicle; 7: No. of filled grains/panicle; 8: Sterility percentage; 9: No. of productive tillers/m^2; 10: Grain yield (kg/ha); 11: Water productivity (kg/m^3); NS: non-significant

Table 4
Effect of system of rice intensification (SRI) practices on yield attributes, yield and water productivity of rice in the dry season (2003)

1	2	3	4	5	6	7	8	9	10	11
T_1	17.9	1.683	6.618	17.99	94.62	76.24	19.43	437	4414	0.2725
T_2	18.4	1.511	6.451	17.78	109.39	79.58	24.51	427	4091	0.3483
T_3	18.8	1.532	6.437	17.87	106.24	74.93	29.47	425	4649	0.3058
T_4	19.2	1.769	6.610	17.96	115.91	80.80	29.43	437	5140	0.4888
T_5	20.1	1.779	15.111	18.71	100.96	75.40	25.23	378	4637	0.2634
T_6	19.8	1.752	15.355	17.96	132.33	76.47	42.21	384	4817	0.2736
T_7	19.7	1.698	16.152	17.79	121.00	81.71	32.45	404	4855	0.3827
T_8	20.1	1.784	16.162	17.97	119.26	83.00	30.40	404	4459	0.2685
T_9	20.1	1.799	15.567	17.35	110.61	81.74	26.10	389	5235	0.3152
T_{10}	20.3	1.656	16.076	17.30	127.58	80.75	36.71	402	4911	0.4287
T_{11}	20.5	1.802	23.071	17.82	159.31	85.46	45.10	370	5143	0.2921
T_{12}	20.8	1.808	25.193	17.73	161.09	90.80	43.49	403	5369	0.3050
T_{13}	20.1	1.820	23.874	17.80	128.16	85.80	33.06	382	5592	0.4408
T_{14}	20.8	1.761	23.939	18.10	136.30	83.18	39.71	383	5078	0.3057
T_{15}	20.0	1.832	23.896	18.19	116.55	86.27	25.98	382	5242	0.3156
T_{16}	20.8	1.736	25.272	17.83	138.26	91.19	35.67	404	5655	0.4937
SEd	0.25	0.016	0.962	1.13	4.91	2.49	4.77	39	158	0.0259
$CD_{P=0.05}$	0.51	0.033	1.962	NS	10.02	5.08	9.73	NS	322	0.0533

For legends see Table 1.

For economizing water, water management in the early stage is very important for the success of seedlings from the dapog nursery. The maintenance of a thin film of water up to 11 days would facilitate better aeration and establishment, leading to increased tiller production. Continuous land submergence is not essential for optimum rice yields, and irrigation could be withheld for two or three days after the disappearance of ponded water without any yield reduction, as observed by Lourduraj and Bayan (1999). However, a marginally lower yield was recorded under the combination of limited irrigation and conventional weeding. This might be due to the low shoot : root ratio caused by the mild stress experienced by the rice plant. This reduction in grain yield was not surprising, as most of the growth and yield components that have a direct bearing on the yield were adversely affected. Boonjung and Fukai (1996) suggested that the variation in DMP at a particular stage due to varying water availability resulted in poor yield components and yield. The combination of limited irrigation and mechanical weeding increased the yield, possibly because it minimised weeds besides improving soil aeration and root pruning (Shad, 1986). Dinesh and Manna (1990) showed that mechanical weeding with a rotary weeder increased the yield in the dry season, but not in the wet season. As weeds were controlled effectively in all the treatments, the results suggest some kind of additional growth-stimulating effect due to mechanical weed control. The effect of mechanical weeding on better soil aeration has been suggested by Uphoff (1999). Improvements in soil aeration by using a rotary hoe help to maintain

better plant health and vigour, thus keeping pest and disease levels below the threshold where the use of pesticides and other agrochemicals is economically profitable. Thus, mechanical weeding paves the way for integrated weed and pest management.

Under SRI practices, the root system is several times longer and deeper, facilitating access to nutrients from a much greater volume of nutrient-poor soil. A larger root system is more likely to capture some of the essential nutrients, such as Zn, Mg, B and other nutrients that are important for plant growth and health. This will provide the plant with balanced nutrition. In addition, mechanical weeding and alternate wetting and drying could also contribute to the biological N fixation dynamism. Deeper root growth encourages higher nutrient absorption and subsequently higher assimilation, which will favour higher yield attributes and yield (Andriankaja, 2001; Rajaonarison, 2001).

The combination of all these factors induced better physiological functions, such as an increased transpiration rate with reduced stomatal diffuse resistance and leaf temperature, with higher relative leaf water content, which had a significant positive correlation with grain yield, and higher levels of nutrient uptake in an integrated manner, which increased the grain yield of rice (Ingram and Yambao, 1998). Rotary hoe weeding primarily facilitated the higher availability of plant nutrients throughout the crop growth period. Nutrients, especially N, promote the synthesis of protein, organic P compounds and carbohydrates and promote better vegetative growth. Phosphorus helps in increasing the test weight and filling percentage, while potassium plays an important role in water uptake and the regulation of its losses through stomatal apertures, improves water relations in plants and thus sustains the grain yield.

As far as economic yield and biological yield are concerned, the combination of factors such as seedling age, spacing, irrigation and weed management with optimum fertilizer management practices complemented each other and boosted the grain yield.

Water productivity

The total quantity of irrigation water used and the frequency of irrigation were higher in the dry season than in the wet season. In the wet season less water was applied due to the well-distributed high rainfall on a larger number of rainy days. However, under water-saving irrigation, the amount of water supplied in the dry season was less than in the case of irrigation to a depth of 5 cm one day after the disappearance of ponded water in the wet season.

Higher water productivity was obtained for the combination of water-saving irrigation and younger seedlings in both crops. This might be due to the fact that younger seedlings mature earlier than conventional seedlings, resulting in higher grain yield and less water consumption under this treatment. These water productivity levels are concomitant with the findings of Bouman and Tuong (2000).

Under the present-day constraints and scarcity of irrigation water the results of the two-year experiments clearly revealed that younger seedlings raised in a dapog nursery and transplanted before the growth of the 4th leaf (14 days old) with a plant density of 16 seedlings m^{-2} (25 × 25 cm) adopting a water-saving irrigation schedule (2 cm on development of hairline cracks) with SRI weeding practices was optimum for the achievement of higher production, productivity and economic returns of rice. Thus, the above SRI practices can be recommended for river and deltaic areas.

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USE OF BARCODES AND DIGITAL BALANCES FOR THE IDENTIFICATION AND MEASUREMENT OF FIELD TRIAL DATA

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The widespread use of digitally-controlled measuring and analytical devices and electronic data collectors, all equipped with microprocessors and linked to computers, has made it possible for on-line data collection to become a routine process. A rational combination of two up-to-date techniques, barcodes and digital balance terminals, linked to an average computer background (Kuti et al., 2003), has proved in practice to satisfy the criteria raised for the up-to-date processing of breeding data at low cost.

This system is an example of how it is possible to reduce costs while processing data more rapidly and reliably and allowing human resources to be utilised more flexibly and efficiently.

The modules (*MvLabel, MvSticker, MvWeighing*) of the program package developed in Martonvásár for the handling and analysis of the data from plant breeding and crop production experiments can also be used independently for the identification of experimental field units (spikes, rows, plots) and for the online handling of weight measurements and analytical data. They provide a simple solution for the design and printing of labels (self-adhesive or plastic) containing barcodes. They make it easier to retrieve the data recorded by digital balance terminals and store them on hard discs, while also helping to unify and synchronise the various parts of the system using barcode readers to identify the measurement data.

Key words: agroinformatics, measurement, barcodes, label printing, breeding, software

Introduction and literary review

Moore's law, which states that the capacity of computers is doubled every eighteen months, has proved true up to the present ever since the revolution in information science began in the 1950s (Moore, 1965). The continuous development achieved in computer science makes its effect felt in manufacturing, communication, marketing, transportation, the health service and, increasingly, in various sectors of agriculture.

The laying out and evaluation of the increasing volume of complex experiments required by today's crop production and breeding research is inconceivable without an ever more complicated data-sensitive background environment. This includes the new, automated generation of experimental field machines, which allow the handling of considerably larger quantities of experimental material. The same is true of the analytical and measuring instruments, all of which now have complicated electronic controls and can be connected up to computers.

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While the evaluation of the experiments is now carried out exclusively by computers, the collection and handling of the increasing number of basic data has not yet been adequately solved in the majority of research units. Traditional manual data recording is labour-intensive, costly, slow and often inaccurate, so it is vital to close the data collection gap, i.e. the discrepancy between data collection and data processing.

It is also obvious that, given the reduction in staff numbers required by efficiency drives, the present system is unsuitable for the routine execution of data collection and evaluation, especially for the large quantities of data involved in the harvesting and sowing cycles so important in breeding.

There is thus a need for application programs (programs planned for the solution of specific tasks directly by the user) suitable for the operation of a comprehensive, up-to-date, economical, automatic data collection system. This system should be capable of collecting and transmitting the data arising in the main fields of research, thus bringing the rate and quality of data collection and data processing to the same level. This means in practice that a whole family of computerised instruments must be designed, which can be used for the input of data originating from various sources (digital balances, laboratory instruments, manual data collectors) into the breeding data model. Naturally, the system will not accommodate older instruments which are unable to transmit data, nor can it be used in situations where measurement and evaluation are entirely the result of manual activity and sensual perception.

During the construction of the system, clearly distinct problems arising during the three main stages of automatic data collection had to be solved:

1. Design of a labelling system suitable for automatic identification

Barcodes are a simple, accurate, cost-saving way of identifying data. The registration of the first barcode patents in the USA (Silver and Woodland, 1952) was followed in 1970 by the Universal Product Code (UPC), suitable for the identification of retail products.

Barcodes became really international when the management of the major companies forming the Article Number Association (ANA) passed a motion regulating the contents of the codes. Instead of the rigid form followed by the UPC (system, manufacturer and product identification number), a "blind" identification number was introduced, where the various digits in the number have no separate meaning based on their position.

Efforts are currently being made by EAN (European Article Numbering) International and the Uniform Code Council (UCC, 1995) in the USA to achieve global acceptance of their standards. Barcode types are described in specifications, such as the USS (Uniform Symbology Specification) Code 128 applied in the present work.

The present barcode types differ in the quantity of data (fixed/variable) they are capable of coding, in the number of characters they use (only digits, 39 characters, 128 characters), and in whether they contain check digits or start-stop and correction characters. The most frequently used linear barcode uses a single row of parallel bars and spaces to code the necessary information. The barcode may also include human-readable printing, so that it can be used for automatic decoding and human reading alike. The use of barcodes ensures reliable identification, reducing the possibility of error to a minimum and allowing real-time data to be read and transmitted by a scanner.

The technology used to read the codes is an automatic identification technique based on illumination and code recognition. When a light source scans the surface containing the barcode, the light rays are reflected by the spaces and absorbed by the bars. The scanner converts the symbols represented by the variously sized bars and spaces in the barcode into an electric signal (with a reading speed of approx. 40 codes per second), after which the digitalised patterns are interpreted by a decoder, converted into the relevant data and transmitted (Marshall, 1991). Transmission may take place to wireless networks using a radio frequency or directly to a computer via a series cable or a keyboard wedge, as if the data had been typed in.

In the course of agricultural research, the barcode labels attached to plot markers, bags, boxes or sacks for use in the field are exposed to intense environmental effects (heat, rain, sunlight, wind, dust, mud). In this case, printers working on the principle of thermotransfer are used, which burn the ink from a special ribbon onto the surface of labels made of cardboard, textile, plastic or metal (Avery, 1985). These labels are extremely durable, withstanding even the most extreme weather conditions. As will be demonstrated below, in other cases (paper bags used in large quantities for laboratory measurements) it is far better to use self-adhesive labels.

2. Production of digitalised measurement data

A protocol for communication between computers and electronic balances or other laboratory instruments was first elaborated in the late sixties by the Electronic Industries Association (EIA) under the name RS232 (RS = Recommended Standard). This was later brought up to date and renamed RS232C (Electronic Industries Association, 1991).

A reduction in the size and, more importantly, price of integrated chips (IC) was needed before the new technique could be used to replace traditional (mechanical) balances by electronic balances, which are quicker, more accurate, and capable of communicating with computers. The same is true of the majority of the new generation of instruments.

In practice, it is advisable to purchase instruments from well-known manufacturers who have a wide range of products, covering all the computer-linked instruments that are likely to be required. The main advantage of using equipment all working on the same or similar principles is that new instruments can be linked up to the system without the need for costly adaptations, as illustrated in Figure 1.

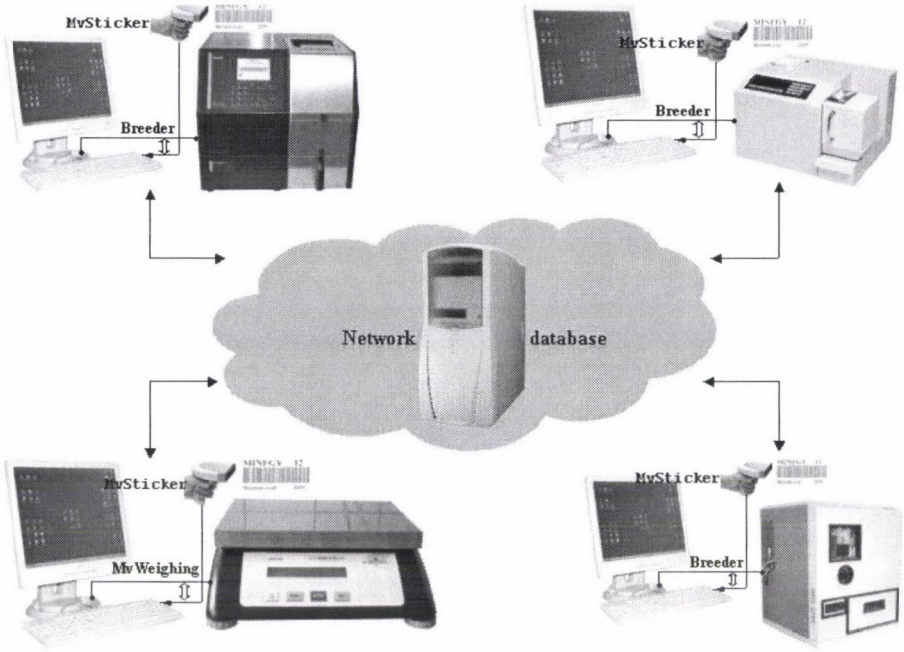

Fig. 1. Scalable weighing system

3. Data collection (computer input)

When the system is intended for independent application for specific tasks (Fig. 2) it must not be forgotten that, due to the differing technical parameters of the electronic devices used for data collection (various communicational interfaces, different command sets, etc.), the planned software will be hardware-dependent. In addition, as the data are, in most cases, transmitted to various types of previously established data models, the software will also be characteristic of the informatic system in question (Láng et al., 2001). It can thus be said that, if the instruments presented here are to be efficiently operated, a tailor-made application which can be directly integrated into the user information system will be required.

The programs available commercially, either as separate products (BalanceLink™ V3.0, © Mettler Toledo) or as part of an operational system (Hyper Terminal, © Hilgraeve Inc., 1999 – part of Windows2000), can be used for the automatic reading of barcodes, and possibly to record the readings from digital balances and other instruments, generally in text files. In some cases, however, it may be necessary to include further interfaces, such as LocalCAN for BalanceLink, or special applications enclosed with the product, which store data and graphs in some special form. This should be clarified prior to purchase.

Fig. 2. Electronic weighing system

Materials and methods

The output of MvSticker, a program designed to plan and print self-adhesive labels of the standard size available commercially, must be linked to a traditional laser or ink-jet printer, so one of the many types of printers now available (HP, EPSON, CANON, etc.) will definitely be required if this program is to be used.

The automatic identification of measurement data is possible using an optical reader/scanner of the CCD (Charge Coupled Devices) type, such as BCH5X49 or BS-L01, offered by the BCL Co. Ltd.

The operational system and the software development devices were chosen with the efficient use of the existing computer background (hardware and software) in mind. Care was taken to ensure that Microsoft® Excel, Microsoft® Access and Microsoft® Word, all part of the Microsoft Office® package (Bott and Leonhard, 1999) run on Windows® (Norton et al., 2000), could be used to display the results.

A program development environment created by Microsoft® for general purposes was used to write the software for the data collection application. The use of Visual Basic® (Jamsa and Klander, 1998; Aitken, 1999) is an advantage to the vast majority of users, who are accustomed to Windows. The default database of Microsoft® Visual Basic® is in the same form as that used by Microsoft® Access, so it was obviously sensible to create the databases forming the backbone of the data model (Szelezsán, 1998) in this format. This means that the results are not only available for the data-handling programs (data recording), but can be viewed, queried and altered using the Access program that is part of the Office® package.

It is important to note that although the universal language SQL (Structured Query Language; ANSI, 1986), which has now been uniformly standardised, is not actually part of Visual Basic®, it is supported by the latter, so this is the primary querying language used for data manipulation in the system described in this paper.

Results

MvSticker printing module for self adhesive labels

While the plastic labels used for the identification of samples originating from field plots and experiments can be printed using the *MvLabel* program designed earlier (Kuti et al., 2003), MvSticker is suitable for the special graphic printing of individually designed self-adhesive labels using a laser printer.

One great advantage of this program is that, in addition to printing alphanumerical characters, it can also print barcode information, if required. It is able to combine information from several columns of structured input data (from Access or Excel) into a single barcode (e.g. Location + Experiment + Plot + Subplot). The barcodes are printed with the aid of a small graphic module incorporated into the program, which is capable of producing the bars and spaces required for the barcode without the need to purchase and install special barcode character sets.

Considering the large number of labels of various types required each year, it was necessary to design a program capable of producing labels directly from the breeding database, according to predetermined label designs. As many labels of very similar type are used from year to year, it is sufficient to create and save the label designs in the first cycle, and the labels needed in subsequent years can be printed with the minimum of effort, after making the necessary modifications using data from the breeding database.

Using the special functions incorporated in the program, complex tasks can be fulfilled, such as the random arrangement of the labels for replicated experiments (Fig. 3, 14) or the use of an auxiliary number to allow two different arrangements of the seeds prepared for sowing (Fig. 3, 4). The order of the labels for the seed bags, on which the source of the seeds is indicated (Previous experiment/Previous plot), is optimised so that all the replications of a particular genotype in the experiments (Fig. 3, 6) and at all locations (Fig. 3, 9) follow each other, making it easier to fill the bags. The labels are then sorted according to locations, and the real sowing order within each location is obtained by sorting on the basis of the auxiliary number, which takes the necessary randomisation into account (Fig. 3, 14). The bags containing the seeds of the different lines are then put into boxes in this order, and from there into the seed drill. Prior to printing the labels the printable content can be visualized in Excel (Fig. 3, 8).

The information required for printing the labels can be entered after opening the program (Fig. 3, 1) from Excel or Access files. The directory path of the open file can be checked on the monitor (Fig. 3, 10).

After choosing the desired label type (in brackets after the rows and columns, Fig. 3, 7), the label sizes (Fig. 3, 13) automatically appear on the screen, thus facilitating the arrangement of the various details within the space available.

When creating new types of labels or modifying a label (Fig. 3, 3) from the existing label files (Fig. 3, 2), it is possible to choose from the database tables automatically presented by the program (Fig. 3, 11) and, after the table is selected, from the various fields of the table (Fig. 3, 12) in order to plan the fields of the new label (Fig. 3, 5).

At any stage in the preparation of the label design it is possible to print a test label (Fig. 3, 15) in the desired row and column of the test sheet in the printer (Fig. 3, 17) to check that the label is satisfactory. After this, all the lines in the data source can be printed according to this label design (Fig. 3, 16).

The information on the label can be designed from any combination of a maximum of 12 different data, including barcodes, alphanumerical characters, or a combination of both. The amount of information which can be printed on the label depends on the size of the label (Fig. 3, 13). The printing of self-adhesive labels generally follows a standard form (Avery Dennison/Zweckform), but if required, other sizes can also be programmed.

The data rows stored in the file can be positioned on the label in the desired form by clicking on one of the 12 label fields (Fig. 3, 5). A new window, designed for the adjustment of the data components, appears on the screen (Fig. 4), in which a list field entitled DataColName (Fig. 4, 2) automatically contains all the column headings of the open data set (Access, Excel). After choosing a column heading, it is possible to select the column containing the data required for the given position on the label. It is then possible to choose barcode or

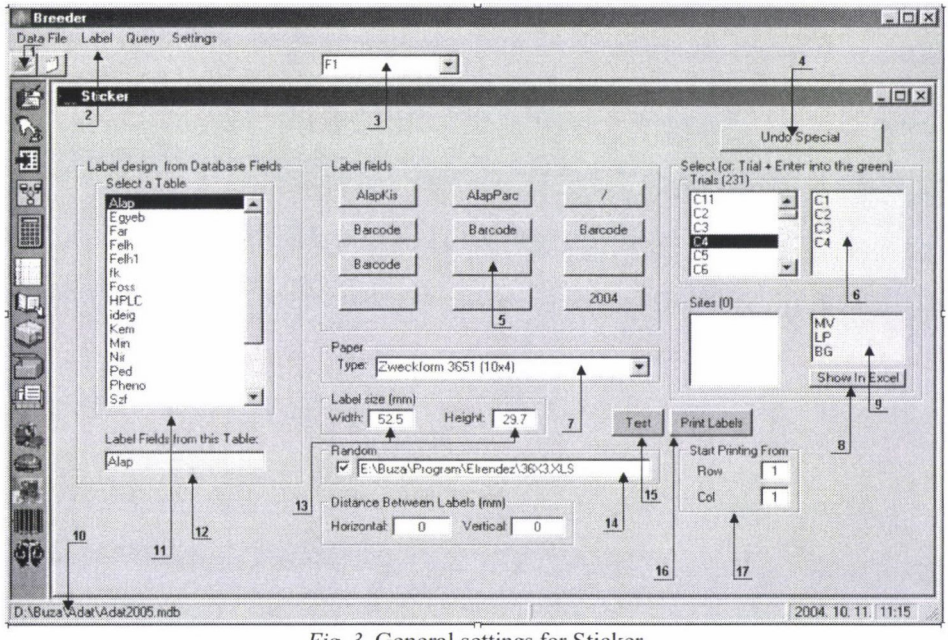

Fig. 3. General settings for Sticker

alphanumerical form (Fig. 4, 1), letter type and size (Fig. 4, 9, 10, 11) and the position on the label (Fig. 4, 6, 7). The positioning of the data on the label is given in mm from the left and top of the label. It is also possible to print constant alphanumerical information on the labels (e.g. year, location, treatment, etc.; Fig. 4, 3) which is not taken from the data files.

If barcodes are to be printed (Fig. 4, 1), it is possible to adjust the size of the barcode (Fig. 4, 4, 5). If several fields on the label are to contain barcodes, these should be given in consecutive fields, in which case the data stored in separate columns in the data file will be automatically included by the program in a complex barcode, separated from each other by spaces. Depending on the requirements of the experiment and the researcher, the barcodes and alphanumerical fields may contain the same or different information. It is expedient for the barcode to contain the information required for identification (e.g. laboratory measurement), while the other information should be printed alphanumerically to facilitate visual identification and evaluation.

If all the fields have been adjusted, it is possible to return to the previous screen (Fig. 4, 12) to set up a new label, or all the adjustments previously made can be deleted (Fig. 4, 8).

The application described here can be integrated directly into the information system of the user, and allows crop production scientists with little computer knowledge to print labels routinely and flexibly.

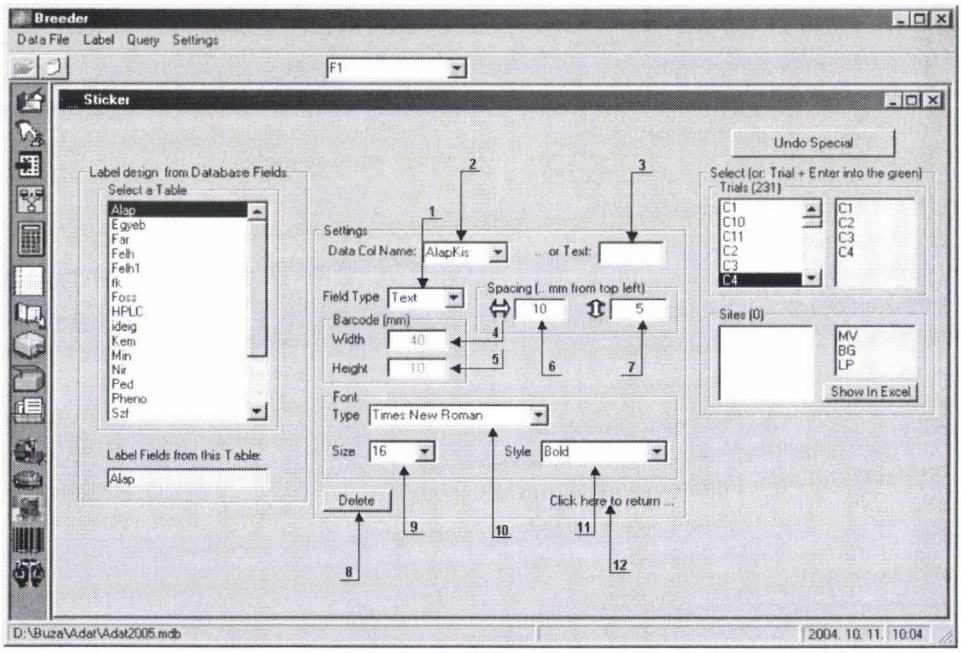

Fig. 4. Sticker field settings

The *MvWeighing* digital balance module

The *MvWeighing* digital balance module allows the data measured by a balance linked up to the computer to be recorded rapidly and correctly online. In its present form it is compatible with digital balance interfaces of the Mettler type, but it can be adapted for use with other makes. In general, the measurement results and the data used to identify the measured values are stored in data structures already created by the user. If such structures do not yet exist, a file must be created in which the data can be stored (Microsoft® Excel or Microsoft® Access). The file used for storage must contain the fields required for identification (e.g. location/experiment/plot number/replication) and a value field for the measured data.

It is not necessary for the user to be acquainted with the settings of the serial line communication required for the coordinated operation of the digital balance and the computer (Fig. 5, 3), so after the empty file, or one already containing other data, is opened (Fig. 5, 1) measurements can begin immediately. The open data file is immediately visible (Fig. 5, 16). In optimum cases, the product to be measured already has a plastic or paper label containing a barcode, from which the barcode reader is able to feed the necessary data into the computer. These data can be checked on the screen (Fig. 5, 6, 7, 8, 9). When the data transmission button on the digital balance is pressed, the measurement data are transmitted and also appear on the computer screen (Fig. 5, 14). Provided both the identification number and the measured data are valid, the identification number and/or the measured data are automatically stored in the open file. The following sample can then be weighed. If the samples are not identified by barcodes, or if the label containing the barcode is damaged to such an extent that it cannot be read, manual data input must be chosen (Fig. 5, 4) and the data must be typed in using the keyboard. Prior to this, the data previously entered into the various fields can be deleted (Fig. 5, 15). In this case the storage of the data is not automatic, so it is necessary to click on the desired button (Fig. 5, 5).

The module can also be used to carry out simple calculations using the measurement data. For instance, if the calculation of thousand kernel mass is selected (Fig. 5, 12), instead of storing the weight of the given number of seeds (Fig. 5, 13), the computer will store the calculated thousand kernel mass value. In the same way it is possible to measure the test weight, or any other parameter that can be calculated from the measurement data with the help of a simple equation.

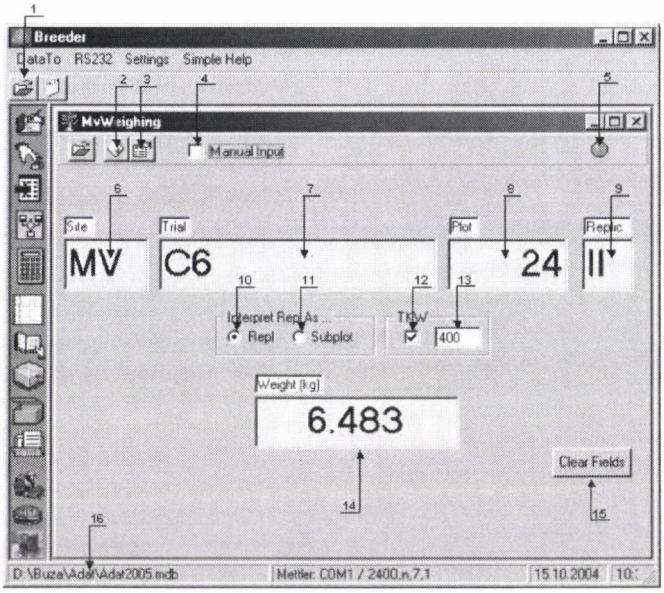

Fig. 5. Weighing settings

Discussion

The two program modules presented in this paper are part of the BREEDER program package developed in Martonvásár to facilitate the registration and analysis of the data from plant breeding and plant production experiments. The most important advantage of this system compared with the previous computerised systems is that some 80% of the manual data input has been replaced by automatic input. The two new modules, in combination with the *MvLabel* program previously described (Kuti et al., 2003), can be linked with instruments capable of communicating with them to collect data which can then be used for statistical analysis, reports, etc. without further manipulation.

In recent years, computer science has undergone rapid development (instruments supplied with microchips, efficient software development tools) and the resulting applications have led to a substantial saving of time, labour and costs in the execution of experiments in crop production and plant breeding.

The system reported here is both up-to-date and economical and can be used in any situation where new data obtained from instrumental measurements need to be integrated daily into a central information system. Potential users will be chiefly researchers working in laboratories and experimental stations, carrying out breeding and field experiments.

The programs have been successfully used in Martonvásár for the last two years for the online storage of data from field and laboratory measurements. A total of more than 200,000 data flow along these channels each year, some of which were previously inserted into the databases by manual input, but the majority of which went unrecorded due to the lack of manual labour.

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MAIZE VARIETIES GROWN IN EASTERN CENTRAL EUROPE BETWEEN 1938 AND 1983

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Several generations of maize breeders contributed to the establishment of genetic resources in Eastern Central Europe by developing open-pollinated varieties, inbred maize hybrids and parental lines successfully grown on large areas and differing from those found in the North American Corn Belt and in other regions of Europe. In some cases they used unusual methods or used known methods in an unorthodox fashion. The Caribbean Flints brought to Hungary from Spain by the Turks in the 16th century played an important role in the development of the Eastern Central Europe genetic resources and dominated Hungarian maize production for nearly four hundred years. In the early 19th century these genetic stocks of Caribbean origin were supplemented by Andean popcorn (Chutucuno Chico, Chutucuno Grande), introduced into Hungary from Italy for human consumption and export purposes and to a lesser extent by Northern Flints (Pennsylvania 8-row). Under the influence of American maize exhibitions in the 19th century, Southern Dents (especially Gourdseed, but also Shoepeg, Hickory King and Tuxpan) and Corn Belt Dents (Queen of the Prairie, Iowa Goldmine, Leaming, and to a lesser extent Funk Yellow Dent) gained ground. In Eastern Central Europe dent varieties were late maturing, so they were crossed, primarily with early-maturing hard flints, and also with early variants of the Caribbean type Old Hungarian Yellow Flint, in order to produce new varieties, which then dominated maize production in the first half of the 20th century. In the early years of hybrid maize breeding, the breeders relied greatly on local, productive, adapted sources.

As the result of hybrid maize breeding in Eastern Central Europe, two distinct gene pools developed, which it is thought could contribute to a further increase in maize yield averages through an improvement in genetic variability. These two gene pools are the Ruma and Mindszentpuszta (MYD) heterosis sources. At least 30 lines of Ruma origin and 19 of MYD origin have been successfully used in the development of commercial hybrids.

From the point of view of breeding early flint × dent hybrids, the European early multi-rowed hard flints, which developed locally, independently of the American Northern Flints, could also be of interest. Less significant varieties and lines that were grown successfully at one time or another could be used as genetic reserves for the development of new variations.

The paper will discuss the varieties popular between 1880 and 1983, providing more detailed data on 13 open-pollinated varieties, 2 variety hybrids, 41 inbred hybrids and 40 successful lines.

Key words: maize, diversity, genetic resources

Introduction

A question frequently considered by maize growers and breeders is how yield averages could be increased. Although many different answers have been given to this question, they have the following points in common: a) the collection of useful sources; b) the preservation of sources of heterosis; and c)

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the continual improvement of initial sources (Russell and Teich, 1967; Russell, 1974; 1983; 1984; 1986; 1991; Brown, 1975; Zuber, 1975; Duvick, 1977; 1981; 1984; 1992; Zuber and Darrah, 1979; Bauman, 1981; Castleberry et al., 1984; Darrah and Zuber, 1985; Smith et al., 1985; 1991; Lamkey and Smith, 1987; Hallauer, 1990; Troyer, 1992; 1996; 1999; 2004; Messmer et al., 1993; Mumm and Dudley, 1994; Radovic and Jelovác, 1995; Dubreuil et al., 1996; Duvick and Cassman, 1999; Frey, 2000; Williams and Hallauer, 2000; Labate et al., 2003). Opinions differ, however, on how sources should be preserved, utilised and improved. Without being specifically stated, it is clear from the works cited above that the majority of authors plan to continue using sources and breeding methods which have been successful in the past.

Maize exists in a wide variety of forms. Excluding races from Africa and Asia, more than 280 races have been described so far and over 10,000 accessions from different variants of these are now preserved in maize gene banks. Although the vast majority of these are primitive races representing the various stages in the evolution of maize through man's intervention, at least ten of them are definitely cultivated races. In spite of this, only two races are known to have been successful: first and foremost the Corn Belt Dent race and, in Europe, the European Flint race. Gerdes et al. (1994) listed 377 open-pollinated varieties of the Corn Belt Dent race which are known and grown under various names. For reasons which are not entirely clear, over the last 80 years hybrid maize breeding has been based chiefly on the variety Reid Yellow Dent (RYD) and to a lesser extent on Lancaster Surecrop and Minnesota 13 (Minn. 13). Other useful sources have served for the development of new variations and have merged into the basic sources of heterosis. This process is still continuing. Lines with mixed genetic backgrounds, such as RYD/Lancaster, Reid/European Flint or Lancaster/European Flint, are now to be found, but it is difficult as yet to judge whether this will lead to the development of new sources of heterosis or, on the contrary, to a gradual reduction in heterosis and an increase in genetic relationship between the genotypes, eventually preventing further increases in yield average being achieved.

One thing, however, appears to be quite clear: in addition to developing new sources of heterosis, sources that are largely unknown but are locally successful may represent important genetic reserves for the hybrid maize breeders of the future.

Many papers have been published on the development of gene pools in Eastern Central Europe and on the results of breeding maize varieties and hybrids (Fleischmann, 1913a; b; 1914; 1916; 1920; 1934; 1939a; b; Teleki, 1926; Villax and Surányi, 1932; Pap, 1950; 1952; 1953; 1954; 1955; Jánossy et al., 1957; Leng et al., 1962; Brandolini, 1968; 1971; Trifunovics, 1978; Németh, 1985; 2003; Balogh, 1988; Berkó and Horváth, 1993; Radovic and Jelovác, 1995; Marton, 2000; 2003; Szundy, 2000; 2003; Gyulavári,2003; Hadi, 2003a; b; 2004; Hadi et al., 2003a; b; c; 2004; Kovács, 2003). These papers suggest that, due to differences in the vegetation period and climate, variety use in Eastern Central Europe differs from that in the American Corn Belt and in other countries of Europe.

The preservation and improvement of successful genetic stocks and the publication of the methods leading to this success could contribute to an increase in maize yield averages not only in the European Corn Belt, but also in other regions.

Materials and methods

The data of seed sales for 13 open-pollinated maize varieties, 2 variety hybrids and 41 inbred hybrids between 1938 and 1983 were processed in the present work. With some modifications, the lists of maize varieties and hybrids were compiled on the basis of Balogh (1988) and Bedő and Veisz (2000), respectively. Seed sales of some of the Martonvásár hybrids registered between 1953 and 1983 did not exceed 0.1% of total sales (Mv SC 635, Mv TC 201, Mv SC 497, Mv SC 394) or were first marketed after the period investigated (BEMA 210, Bermador, etc.), while the papers quoted did not include Mv DC 58, Mv TC 521, Mv TC 651, Mv DC 350 or Mv SC 587 which, although they were not registered, were licensed for seed multiplication and enough seed was sold for the sowing of 329,151 ha (1.6%).

The data on seed sales per variety were taken from the compilation of Balogh (1988), who was employed in important posts in the seed industry for 40 years and who prepared an accurate seed balance each year. In the majority of cases the data are presented as the percentage of the total sowing area, but in some cases information is given on the size of the sowing area or on the quantity of seed sold.

As the totals were given in tons for each variety, the total seed sales were transformed into per hectare values based on the total sowing area in the given year. Naturally, this transformation led to a slight decrease in the accuracy. A further source of inaccuracy could be the fact that Balogh (1988) gave the proportion of open-pollinated varieties as the quantity of certified seed sold on the market, which was corrected for the real sowing area per variety per year.

An earlier paper discussed the contribution made to Hungarian maize production by 22 hybrids developed from lines originating from the Mindszentpusztai Yellow Dent (MYD) variety (Hadi et al., 2004). It appears, however, that Hungarian maize seed sales can be characterised more accurately by processing the data of the 41 Martonvásár-bred hybrids registered and grown between 1953 and 1983.

The relative contribution of various heterosis sources was calculated according to the guidelines of Troyer (1999; 2000). The lines were traced back to the sources. The relative proportions of the lines and sources in each hybrid were multiplied by the sowing area of the hybrid, and the size of the area sown was summed for each line (heterosis source).

The probable or known breeders of the open-pollinated varieties listed in the paper, together with the date of breeding, are included in Table 1.

The breeders of the Martonvásár inbred hybrids were first Endre Pap, and later István Kovács, András Csetneki, Márton Herczegh, Károly Kovács, István Manninger and Bertalan Dolinka.

Results

The years 1938–1983 can be divided into two main periods on the basis of the maize varieties used in Hungary. Although the first period, when registered maize varieties bred from local sources played a dominant role in production, really began in the early 1900s, records of the real seed sales for each variety were only kept from 1938 onwards. The end of this period came when inbred hybrids became dominant and the use of open-pollinated varieties declined.

Table 1
Origin of open-pollinated varieties grown in Hungary (1938–1962)

Variety name	Origin	Breeders
Fleischmann Mezőhegyesi = F.M.H. ("F" Mezőhegyesi Y.D.)	"F" Aranysárga lófogú (="F" Golden Yellow Dent) (from: mother plant No 122 Rumai/1908, source: Livingstone's Early Golden × Early Bánáti Flint)	R. Fleischmann (1908–1934) F. Szüllő (1930–1962)
Fleischmann Korai = "F" korai ("F" Early Y.D.)	"F" Aranysárga lófogú (="F" Golden Yellow Dent) (from: mothern plant No. 122 Rumai/1908, source: Livingstone's Early Golden × Early Bánáti Flint)	R. Fleischmann (1908–1951) J. Lelley–Z. Sarkadi (1952–1962)
Mindszentpusztai fehér = M.p.f. (Mindszentpusztai White Flint)	Padova White Flint (Italy) (source: Guatemalan conical flints)	E. Pap (1917–1956) I. Kovács (1957–1962)
Mindszentpusztai sárga lófogú = MPS (Mindszentpusztai Y.D.)	Chester Leaming	E. Pap (1917–1956) I. Kovács (1957–1962)
Pettendi Aranyözön sárga lófogú = Aranyözön (Pettend Golden Flood Y.D.)	Unknown American Y.D. × Putyi	E. Mecsér (1921–1944) H. Taróczi (1945–1950) O. Gyulavári (1951–1962)
Bánkuti lófogú = Bánkuti (Bánkuti Y.D.)	Pignolettó from Béllye × (Bristol; Queen of Prairie Mastodon; Unknown white dent) Pignolettó (Italy) from Chutucuno Grande (Chile)	L. Baross (1895–1928) F. Beke (1938–1962)
Putyi	Old Hungarian Yellow Flint × Cinquantino BC(2) (Old Hungarian Yellow Flint from Caribbean Flints, Cinquantino (Italy) from Chutucuno Chico (Chile)	P. Németh (1856–1879) J. Balogh (1880–1890) E. Lubinszky (1897–1910) E. Mesterházi (1908–1924) E. Székács (1915–1938) V. Kopeczky (1932–1949)
Szegedi sárga lófogú= Szegedi (Szegedi Y.D.)	Funk Yellow Dent (Early selection)	Á. Teleki (1909–1930) L. Kesztyüs (1914–927) F. Somorjai (1930–1962)
Martonvásári F.B. (M.F.B.)	"F" Early × Pennsylvania 8-row	B. Friedrich (1922–1962)
Iregi 12 hetes (Iregi 12-week = Mauthner 12-week)	North Dakota White Flint	E. Kurnik – A. Oberritter (1939–1951)
Red King	King Philip × Unknown Y.D.	Unknown
"M" siló (silage)	Mindszentpusztai White Flint × White Cinquantino	A.G. Manninger (1934–1960)
"Vas" siló (silage)	Several Open Pollinated Varieties × Pettend Golden Flood	I. Vas (1935–1960)
Óvári 1 variety hybrid (Ó.1.)	Mindszentpusztai white flint × Martonvásári F.B. (M.p.f. × M.F.B.)	L. Berzsenyi–Janosits (1948–1953)
Óvári 5 variety hybrid (Ó.5.)	"F" Early × Pettend Golden Flood ("F" korai × Aranyözön)	L. Berzsenyi–Janosits (1948–1953)

To the best of our knowledge, in the early years of the century, up to 1938, large areas were sown to the hard flint varieties Béllyei, Alcsúti, Esterházai, Sátorhelyi Pignolettó, Legkorábbi Székely (Earliest Székely) and Putyi, the soft flint varieties Lészai, Rábaközi, Zsombolyai, Algyógyi, Lapusnyaki and Korai Bánáti (Early Bánáti), the hard dent varieties Késői Bánkúti Lófogú (Late Bánkúti Dent) and Korai Bánkúti Lófogú (Early Bánkúti Dent), and the soft dent varieties Gyérei-Dudás Fehér Lófogú (Gyérei-Dudás

White Dent), Szalontai Lófogú (Szalontai Dent), Iowa Goldmine, Queen of the Prairie and Illinois Champion (Hadi et al., 2003b). However, only the open-pollinated varieties listed in Table 2 were found in the records of seed sales.

The sowing area of the open-pollinated varieties is given in Table 3. It is worth noting that the sowing area of the "F" (Fleischmann) varieties, namely "F" Aranysárga Lófogú (Golden Dent) (derived from the mother plant Rumai No. 122), its derivative "F" Mezőhegyesi Sárga Lófogú (Mezőhegyesi Yellow Dent), and "F" Korai Sárga Lófogú (Early Yellow Dent), was nearly 13.5 million hectares between 1938 and 1962. Rudolf Fleischmann, the breeder of the "F" varieties, started breeding maize in Ruma (Serbia) in 1908 from a mixed or accidentally/natural hybrid population of the local Gourdseed type variety Korai Arany (Early Golden) and the variety Korai Bánáti Flint. After Fleischmann moved his stock to Kompolt (Hungary) in 1919, his variety was registered in Hungary in 1923 under the name "F" Aranysárga Lófogú. It is estimated that the area sown to "F" varieties was over 5 million hectares between 1923 and 1938, and as much as 18.5 million hectares over the whole lifespan of the varieties. Although no exact information is available, it is clear that the varieties Rumai Yellow Dent, Vukovári Yellow Dent and Béllyei Yellow Dent, multiplied by Fleischmann from the mother plant Rumai No. 122 in Ruma and later improved by Yugoslav breeders, were just as successful in Yugoslavia as the "F" varieties in Hungary. This is confirmed by the fact that at least 22 first-cycle lines and a total of 30 lines used in commercial hybrids were developed from varieties originating from Rumai No. 122. Of these, GK 13, GK 22, GK 42 and Be03b were widely used in Hungary (Hadi et al., 2003a).

Table 2
Sowing area of open-pollinated maize varieties in Hungary (1946–1962)

Variety	Sowing area (%)																
	'46	'47	'48	'49	'50	'51	'52	'53	'54	'55	'56	'57	'58	'59	'60	'61	'62
"F" Mezőhegyesi	35	30	26	26	26	27	27	20	20	19	20	21	17	12	7	5	3
"F" Korai	20	21	22	23	24	26	27	25	24	23	22	24	21	13	8	8	5
Mindszentpusztai White	5	7	9	9	8	10	11	7	6	4	2	2	2	1	–	–	–
Mindszentpusztai Yellow Dent	10	12	11	12	12	11	12	10	8	7	6	5	5	3	2	–	–
Aranyözön Yellow Dent	10	12	13	11	10	9	9	7	9	10	8	12	16	13	10	8	5
Bánkuti Dent	5	6	6	5	6	5	5	2	2	2	3	3	3	2	1	–	–
Putyi	10	5	6	5	5	4	1	–	–	–	–	–	–	–	–	–	–
Szegedi Yellow Dent	–	–	–	–	–	–	–	5	6	8	8	5	9	5	4	5	2
Martonvásári FB	–	–	–	–	–	–	–	7	7	6	5	4	5	3	2	–	–
Iregi 12 hetes (12-week)	–	–	–	–	–	–	–	2	2	2	2	1	1	1	1	–	–
Red King	–	–	–	–	–	–	–	4	6	8	5	4	4	3	–	–	–
"M" silage	–	–	–	–	–	–	–	–	–	–	0.5	0.5	0.5	0.5	0.5	–	–
"Vas" silage	–	–	–	–	–	–	–	–	–	–	0.5	0.5	0.5	0.5	0.5	–	–
Óvári 1	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
Óvári 5	–	–	–	–	–	–	–	–	3	6	18	11	7	12	–	–	–
Other varieties	5	7	7	9	9	8	8	10	7	5	3	4	6	3	3	7	5
Inbred hybrids	–	–	–	–	–	–	–	–	–	–	1	3	3	28	56	65	80

Table 3
Distribution of sowing area of open-pollinated varieties grown in Hungary (1938–1962)

No.	Variety	Year of registration	Years when cultivated	1938–1945*		1946–1962*	
				ha	%	ha	%
1	"F" Mezőhegyesi Y.D.	1942	1938–62	3,255,000	35	4,083,770	23.3
2	"F" Korai Y.D.	1930	1938–62	1,860,000	20	4,301,031	24.5
3	Mindszentpusztai White	1928	1938–59	465,000	5	1,006,330	5.7
4	Mindszentpusztai Y.D.	1928	1938–60	930,000	10	1,529,718	8.7
5	Aranyözön Y.D.	1938	1938–62	930,000	10	2,043,785	11.6
6	Bánkuti Dent	1943	1938–60	465,000	5	689,989	3.9
7	Putyi	1941	1938–52	930,000	10	441,185	2.5
8	Szegedi Y.D.	1951	1953–62	–	–	722,785	4.1
9	Martonvásári FB	1951	1953–60	–	–	484,965	2.8
10	Iregi 12 hetes (12-week)	1951	1953–60	–	–	149,100	0.8
11	Red King	–	1953–59	–	–	423,433	2.5
12	"M" silage	1954	1956–60	–	–	32,858	0.2
13	"Vas" silage	1951	1956–60	–	–	32,858	0.2
14	Óvári 1	1953	1953	–	–	11,612	0.1
15	Óvári 5	1953	1954–59	–	–	723,552	4.1
16	Other varieties (not specified)	–	–	465,000	5	1,324,746	7.5
	Total:			9,300,000	100	17,548,852	100.0

*Total estimated sowing area

Mindszentpusztai Yellow Dent (MYD) was an outstanding open-pollinated variety, grown on around 10% of the sowing area each year. It was grown on 2.5 million hectares in Hungary between 1938 and 1962, and on at least 3.5 million hectares in all. It was from this variety that Endre Pap developed lines 01, 014, 0118b and 156, and probably also line 0118a, between 1933 and 1945; these lines had a great influence on the breeding of hybrid maize in Hungary, and in Europe as a whole (Hadi et al., 2003c; 2004).

The variety Pettendi Aranyözön was also grown on 10% of the sowing area for a long period. Its excellent combining ability allowed it to be used in breeding variety hybrids (Óvári 5, Óvári 7), while a line developed from it was also used successfully in the inbred hybrid Kollektív 1 (Gyulavári, 2003).

The variety Mindszentpusztai White occupied 5–6% of the sowing area. It was used in breeding the variety hybrids Óvári 1, Óvári 3 and Óvári 4, the variety-line hybrid Mv 26, and the F 5 fixed line, a very early silage maize developed as the result of German-Polish-Hungarian breeding cooperation and grown in Germany and Poland.

With the exception of Putyi (Szv 367), Szőregi Lófogú (Szőregi Dent; Szv 293) and Bánkúti korai (Bánkuti Early; B 125), other popular open-pollinated varieties were important from a production point of view, but made little contribution to the breeding of hybrid maize.

The significance of the open-pollinated varieties bred and cultivated in Hungary lies in the fact that they had excellent combining ability (Fleischmann,

1939b; Jánossy et al., 1957, Berzsenyi-Janosits, 1958; Gyulavári, 2003) and an origin quite different from the varieties grown in the American Corn Belt and in other parts of Europe (Table 1), making them important sources of heterosis for hybrid maize breeding in the past (Hadi et al., 2003). Nowadays, however, these original sources fall far behind modern hybrids as regards their value in breeding.

The hybrids bred in Martonvásár played an important role in Hungarian maize production between 1956 and 1983 (Table 4), making up 56% of the total seed sales during this period. This level of representation was similar to that achieved by the Pioneer hybrids in the American Corn Belt (Troyer, 1999; 2000). It is noteworthy that nearly 94% of the seed was developed using lines originating from the Mindszentpusztai Yellow Dent variety.

Table 4
Contribution of Martonvásár maize hybrids to Hungarian maize production (1956-1983)

Year	No. of varieties or hybrids grown	Maize sowing area in Hungary (ha)	Sowing area (ha)	Market share (%)	Sowing area (ha)	Market share (%)
			of Martonvásár hybrids		of hybrids developed from MYD lines	
1956	15	1,162,925	11,629	1.0	11,629	100.0
1957	15	1,346,575	40,398	3.0	40,398	100.0
1958	15	1,304,000	39,120	3.0	39,120	100.0
1959	15	1,358,000	380,240	28.0	380,240	100.0
1960	15	1,401,000	868,620	62.0	868,620	100.0
1961	9	1,304,000	873,680	67.0	873,680	100.0
1962	12	1,288,000	1,030,384	79.9	1,030,384	100.0
1963	9	1,288,847	1,220,360	94.5	1,220,360	100.0
1964	6	1,208,000	1,206,475	99.9	1,206,475	100.0
1965	8	1,217,980	1,192,424	97.9	1,192,424	100.0
1966	8	1,237,000	1,199,892	97.0	1,199,892	100.0
1967	10	1,237,000	1,159,069	93.7	1,159,069	100.0
1968	9	1,258,441	1,097,325	87.2	1,097,325	100.0
1969	26	1,255,140	1,096,375	87.3	1,065,552	97.2
1970	30	1,188,831	1,014,139	85.3	901,110	88.9
1971	41	1,321,000	1,130,010	85.5	923,379	81.7
1972	32	1,392,000	1,279,208	91.9	1,113,816	87.1
1973	44	1,460,764	1,185,199	81.1	1,066,212	89.9
1974	47	1,461,493	1,109,211	75.9	984,987	89.0
1975	88	1,412,540	876,453	62.1	752,861	85.9
1976	72	1,339,000	621,296	46.4	530,244	85.3
1977	59	1,281,000	561,078	43.8	491,904	87.7
1978	51	1,283,000	418,258	32.6	346,410	82.8
1979	62	1,352,000	355,340	26.3	267,613	75.3
1980	62	1,229,000	142,687	11.6	138,877	97.3
1981	73	1,163,000	97,695	8.4	90,717	92.9
1982	69	1,130,000	70,010	6.2	46,330	66.2
1983	92	1,107,000	27,655	2.5	8,836	31.9
Total		35,987,536	20,304,230	56.4	19,048,464	93.8

A total of 28 hybrids involving the five lines of Mindszentpusztai Yellow Dent were registered between 1953 and 1983, but they were not all cultivated at the same time. After 1973 the number of rival hybrids was over 40 each year. From 1975 onwards the previously closed Hungarian seed market became open and although the yield of hybrids developed from MYD lines was competitive with that of foreign varieties, their stalk strength was deficient when the plants were overripe, while the grain moisture content at harvest was above average. When grown at high plant density (75–85 thousand plants/ha) there was an increase in the number of barren plants, and as mechanical harvesting became general these varieties were grown on an ever smaller area (Marton 1999). This change of genotype, however, affected not only hybrids developed from MYD lines, but also other Martonvásár, Hungarian, European and American hybrids. This period was characterised by high rates of nutrients, high plant density, combine harvesting and an increase in drying costs.

The hybrids grown in Hungary between 1956 and 1983 can be characterised by their pedigrees (Table 5), their lifespan, and the total quantity of seed marketed during their lifespan. Among the listed hybrids, the early maturing hybrids BEMA 250 and BEMA 210 were sold not only in Hungary, but also in East Germany and Poland, though the exact distribution of the sales was not recorded. The first hybrids registered in Europe, Mv DC 5 and Mv DC 1, were sown on the greatest areas, but the hybrids Mv DC 602, Mv DC 59, Mv MC 40 and Mv TC 596 were also grown on an area of around 1 million hectares or more. The variety line silage hybrid Mv 26 remained on the market for 19 years and the grain maize hybrid Mv DC 5 for 16 years. All the hybrids mentioned above contained one or more lines originating from MYD. Mv DC 5, for instance, contained three. It is clear from the pedigrees of the hybrids that lines 01 and 014 combined extremely well with lines 156 and 0118b. The MYD lines also combined well with numerous lines of other origin. Among the hybrids which did not include MYD lines, Mv DC 460 was the most popular (400,000 hectares). Although this hybrid did not contain MYD lines, other local lines were included in its pedigree, among which Be03b, originating from Béllyei Yellow Dent, and B 125, originating from Bánkúti Korai, made a substantial contribution to the excellent adaptability and popularity of the hybrid. Among the other local lines, HMv 850, a distant relation of HY-2, was the most popular.

The very low frequency with which BSSS and Lancaster lines were used is somewhat surprising. Among the BSSS lines, only B 14 was used in the development of a popular hybrid, Mv SC 580 (477,000 ha). Very little seed was sold from the hybrid Mv SC 484, which contained the line A 632, while Mv SC 497, which also contained this line, never went on the market. Mv SC 434, which included improved versions of lines A 632 and B 37, was somewhat more popular, and continued to be sold beyond the period examined.

Table 5
Pedigree of Martonvásár hybrids grown in Hungary (1956–1983)

Hybrid	Pedigree	Year of registr.	Cultivated		Total seed sales (in ha)
			between	No. of years	
Mv DC 5	(0118b×156)×(C5×014)	1953	1956–71	16	2,796,479
Mv DC 1	(WF9×M14)×(C5×014)	1955	1960–71	12	4,497,800
Mv MC 39	[(WF9×M14)×(C5×014)×(0118b×156)]	1957	1960–63	4	157,058
Mv MC 40	[(A96×A34)×(0118b×156)×(Min6×01)]	1959	1961–73	13	1,497,012
Mv DC 42	(Iregi×L17)×(Min6×01)	1960	1962–67	7	43,262
Mv DC 57	(0118b×156)×(A96×A34)	–	1962–64	3	22,388
Mv DC 58	(0118b×156)×(Min6×01)	–	1963	1	25,776
Mv DC 48	(C5×WF9)×(0118b×156)	1961	1962–69	8	758,616
Mv 26	(C5×014)×Mindszentpusztai White	1961	1965–83	19	660,804
Mv DC 59	(C5Cms×N6)×(0118b×156)	1962	1965–75	11	1,854,216
Mv DC 602	(WF9×N6)×(C5×014)	1964	1967–77	11	2,430,825
Mv DC 502	(156×OH43)×(C5×014)	1966	1968–70	3	26,184
Mv SC 520	(156×N6)×(C5×014)	1968	1969–81	13	509,700
Mv TC 521	(C5×N6)×B125	1968	1969–71	3	50,896
Mv SC 530	156×N6	1968	1969–78	10	604,667
Mv SC 620	WF9×N6	1968	1969–76	8	254,569
Mv TC 651	(WF9×N6)×C103	1969	1971–73	3	34,016
Mv TC 290	(0118a×W153R)×EP1	1970	1972–77	6	68,429
Mv SC 370	156×A90	1970	1972–74	3	105,898
MvTC 431	(156×N6)×C5	1970	1968–78	11	620,234
Mv TC 540	(Be03b×N6)×B125	1970	1970–76	7	190,880
Mv SC 570	C5×N6	1970	1972–74	3	42,368
Mv TC 596	(156×N6)×HMv850	1970	1971–82	12	953,230
Mv TC 610	(C5×N6)×HMv850	1970	1969–72	4	64,555
MvTC 281	(0118a×A90)×EP1	1971	1972–75	4	55,911
Mv DC 460	(B125×B18/4)×(Be03b×N6)	1971	1973–80	8	405,366
Mv SC 660	N6×C103	1971	1970–75	6	30,931
Mv MSC 262	(0118a×0118aR2)×EP1	1972	1974–76	3	17,121
Mv SC 380	156×W153R	1972	1971–78	8	410,623
Mv SC 580	156×B14	1972	1973–81	9	477,813
Mv SC 587	A374h×CE187	1972	1973–75	3	27,717
BEMA 250	(0118aR2×W153R)×(Dbe19×DBe42)	1974	1976–82	7	179,549
Mv SC 405	156×B18/4	1974	1973–80	8	173,425
Mv DC 350	(0118a×A90)×(156×W153R)	–	1975	1	11,300
Mv MSC 342	(HMv480×W153R)×A90	1976	1976–79	4	36,299
Mv SC 424	156×F564	1976	1978–79	2	3,918
Mv SC 429	156×HMv401	1976	1975–81	7	124,584
Mv TC 296	(0118aR2×W153R)×HMv404-C	1978	1979–83	5	40,888
Mv SC 484	F564×A632	1978	1979–83	5	19,865
BEMA TC 210	(F7CmsC×F2)×CM7/Mv	1980	1981–83	3	19,151
Mv SC 434	HMv403×MA61A47D	1981	1981–83	3	15,623
Total:					20,304,230

Among the Lancaster lines, C 103 was used in two hybrids, Mv TC 651 and Mv SC 660, of which sufficient seed was sold for 65,000 hectares in all, while for Mv DC 502, which contained line OH 43, this figure was 26,000 hectares. No Martonvásár hybrids containing lines A 619 or Mo 17 were registered between 1953 and 1983, despite the large number of hybrids entered for state trials. By contrast, hybrids developed using lines originating from the Wisconsin line C5 = W 23 or the Nebraska line N 6 were successfully grown. The lines used in Hungary differed significantly from those used in the Corn Belt. This can probably be attributed to the shorter vegetation period, the considerably drier continental summer, the wetter autumn, favourable for the spread of ear rot, and the lower rates of fertiliser applied.

The proportion of each inbred line in individual hybrids was multiplied by the sowing area of the hybrids and totalled for each line (Table 6). The most popular line in Hungary between 1956 and 1983 was C 5 = W 23, used with a frequency of 18.7%. Other popular lines were 156 (15%), 014 (13.5%), N 6 (11.2%) and WF 9 (10.2%). It is interesting to note that four of the five MYD lines in the list had a frequency of over 1%, averaged over almost 20 years. Among the local lines, HMv 850 (2.51%), B 125 (1.09%), B 18/4 (0.93%) and Be03b (0.73%) were also used fairly frequently. It is clear from the table that flint lines were no more popular than the BSSS and Lancaster lines. The probable reason for the low contribution of flint lines is that most silage maize grown in Hungary belongs to the FAO 400–500 maturity group, while the FAO 200 hybrids are harvested with low grain moisture content as early grain maize.

During the period 1953–1983 a total of 49 hybrids developed using the five original and two improved MYD lines were registered in Hungary (Table 7), including several containing more than one MYD line. These were sown on a total of almost 8 million hectares. The most popular line was 156, used in 20 registered hybrids, followed by 0118a (8 hybrids), 014 (7), 0118b (7), 01 (3), 0118aR2 (3) and HMv 404-C (1). Original and improved lines derived from MYD contributed to the success of hybrid maize breeding not only in Hungary, but also in France, Germany, Moldova, Bulgaria and Canada (Hadi et al. 2004). The improved MYD lines are still important sources of heterosis in maize breeding.

Between 1953 and 1983, seed sales of hybrids developed using the four major sources of heterosis were sufficient for over 17 million hectares (Table 8), representing 85% of all the hybrid seed listed. The most popular heterosis source was MYD (38.84%), followed by Golden Glow (18.6%) and Hayes Golden (11.37%). These heterosis sources were never particularly popular either in the American Corn Belt or in other European countries, while Reid Yellow Dent, which was used with a frequency of little more than 16%, had a quite different composition to that used in the North American Corn Belt.

Table 6
Frequency with which inbred lines were used in Martonvásár maize hybrids (1956–1983)

Line	Origin	Seed sales, corrected with the genetic contribution of the line (ha)	Frequency of line use (%)
C 5	W 23 = Golden Glow	3,791,562	18.67
156	MPS C_0	3,035,966	14.95
014	MPS C_0	2,,750,080	13.54
N 6	Hayes Golden	2,276,511	11.21
WF 9	Wilson Farm Reid	2,077,230	10.23
0118b	MPS C_0	1,590,761	7.83
M 14	BR 10 × R 8	1,144,082	5.63
HMv 850	U.W.W. 30 (HY-2 rel.)	508,893	2.51
01	MPS C_0	391,512	1.93
Min. 6	Min. No. 13	385,068	1.90
M.p.f.	Mindszentpusztai White Flint	330,402	1.63
W153R	(I.a.153 × W8) × I.a 153	289,428	1.43
B 14	BSSS C_0	238,907	1.18
B 125	Bánkuti Early Dent	222,229	1.09
A 96	64 × H	192,724	0.95
A 34	Rustler (C 15)	192,724	0.95
B 18/4	A374h × A118, A374h rec.*	188,054	0,93
Be03b	Béllyei Yellow Dent	149,062	0.73
EP 1	Lizargarote	70,730	0.35
HMv 401	N6 × M5226, N6 rec.*	62,292	0.31
0118aR2	0118 mutant	59,389	0.29
A 90	64 × 15-28	56,137	0.28
Dbe 19	Pommermais	44,887	0.22
Dbe 42	Sammerlaktion Lübears	44,887	0.22
0118a	MPS C_0	38,190	0.19
C 103	Lancaster Sure Crop	32,474	0.16
HMv404-C	(156×C131A)×156 BC_3	20,444	0.10
A 374h	A 374 rec.*	13,858	0.07
CE 187	C.I. 187-2 rec.	13,859	0.07
Iregi	Iregi 12-week flint	10,815	0.05
L 17	Poland O.P.V. (Wielkopolanka?)	10,815	0.05
F 564	F 564 rec.*	9,932	0.05
A 632	(Mt42×B14)×$B14^3$	9,932	0.05
CM7/Mv	CM 7 rec.*	9,575	0.05
HMv 480	W153R rec.*	9,075	0.04
HMv 403	A632×M5226, A 632 rec.*	7,811	0.04
MA61A47D	B37 × W 79A	7,811	0.04
OH 43	OH40B×W8	6,546	0.03
F2	Lacaune	4,787	0.02
F7	Lacaune	4,788	0.02
Total:		20,304,230	100.00

*rec. = recovery

Table 7
Contribution of MYD lines to the seed produced and sold in Hungary (1956–1983)

Line	No. of cultivated hybrids	Seed sales, corrected with the genetic contribution of the line	
		(ha)	(%)
156	20	3,035,966	38.50
014	7	2,750,080	34.87
0118b	7	1,590,761	20.17
01	3	391,512	4.96
0118aR2	3	59,389	0.75
0118a	8	38,190	0.48
HMv404-C	1	20,444	0.26
Total:	49	7,886,342	100.00

Table 8
Basic sources of heterosis in Hungary between 1953 and 1983

Source of heterosis	Total seed sold	
	(ha)	(%)
Mindszentpusztai Yellow Dent	7,886,342	38.84
Golden Glow	3,791,562	18.67
Reid Yellow Dent	3,257,402	16.04
Hayes Golden	2,307,657	11.37
Total:	17,242,963	84.92

Discussion

Pollmer (1971) mentioned 279 maize varieties bred or collected in Hungary, while the variety collection of the Agrobotanical Institute in Tápiószele (Index Seminum, 1973) contained 29 dent and 21 flint varieties. Gerdes et al. (1994) reported finding 377 dent and 213 flint varieties in the North American Corn Belt, of which 47 (12.5%) dent and 10 (4.7%) flint varieties were developed from crosses designed in the Corn Belt. These authors make no mention of the origin of the Hungarian varieties. Of the 13 open-pollinated varieties successfully bred in Hungary from 1938–1962 nine (70%) were bred from hybrid sources. This was probably due to the fact that the vegetation period of varieties from the American Corn Belt and from Italy was too long to ensure their maturing reliably in Hungary. Breeders attempted to solve this problem in two ways, either by selecting for earliness over several generations (Szegedi Yellow Dent, Mindszentpusztai White, Mindszentpusztai Yellow, "F" Early) or by crossing local, early-maturing flint varieties with the late dent varieties to develop basic breeding stock (Szentkirályi, 1881; Grábner, 1908; 1916; 1922; Péterfy, 1912a, b; 1913; Anonymous, 1914; Teleki, 1926). The varieties Cinquantino and Pignoletto, which reached Hungary from Chile via Italy in the early 1800s, were used most frequently as early donors. These were daylength-

insensitive, chilling-tolerant varieties with multiple ears and reddish-brown kernels of the hard flint type, suitable for making popcorn (Chutucuno Chico, Chutucuno Grande). These varieties were improved in Hungary for early flowering, cylindrical ear shape, number of kernel rows, kernel length and prolific ears for at least 60 years. Early, improved variants of the variety Old Hungarian Yellow (8–12-row) Flint, which was brought to Hungary in the 1500s and is probably of Early Caribbean origin, were also used in crosses designed to develop basic breeding stock.

Early hard flints were crossed not only with dent varieties, but also with early variants of Old Hungarian Yellow Flint, giving rise among others to the varieties Putyi (Péterfy, 1912b; 1913) and Legkorábbi Székely (Szentkirályi, 1881). The latter was an extra early maturing variety with great yield potential and was successfully cultivated in Hungary for forty years. It was also introduced and grown in other European countries. Several 100 tonnes of seed were annually exported to France, Germany, Austria, Bohemia, Poland and Russia between 1890 and 1910. In recognition of its excellent performance, this variety was given many awards and won the Grand Prize at the World Fairs in Brussels in 1897 and Paris in 1900. The germplasm of this variety probably contributed to the development of the European, multi-rowed, early flints, which differed in origin from the Northern Flints (Hadi et al., 2003b).

Open-pollinated varieties which were successfully cultivated, such as Bánkúti (late) dent, the "F" varieties, Aranyözön and the Mindszentpusztai varieties, were developed partly by crossing Hungarian sources and partly as the result of improvement in Hungary over many generations. As a result, their high yield potential was combined with early maturity and good adaptability, while their diverse origin contributed to the excellent combining ability of the open-pollinated varieties themselves and of lines derived from them. Hybrid maize breeding in Hungary was based on MYD, which was probably of Leaming origin, while in Yugoslavia it was based on varieties developed and multiplied from the mother plant Rumai No. 122, a genetic mixture of Livingstone's Early Golden, which was of the Gourdseed type, and Korai (Early) Bánkúti, which was of the Caribbean type.

Two quite different breeding methods were used in the development of the most popular open-pollinated varieties, both of which indirectly promoted the rapid formation and fixation of variety characteristics.

The variety Legkorábbi (Earliest) Székely was bred by Árpád Szentkirályi after a long period of mass selection for ear and kernel types. Only kernels from ears very close to the selection criteria (similar for ear length, number of kernel rows, kernel type, kernel colour, ear mass, etc.) were used to create the elite seed mixture; ears that did not satisfy these criteria were not regarded as true to the variety. This method of mass selection was also used in the North American Corn Belt, since Teleki (1926) reported that the Funk Yellow Dent variety used as a source in developing Szegedi Yellow Dent was maintained in this way.

Stabilising selection of this type was also used widely in Hungary, for instance in the breeding and variety maintenance of the varieties Lapusnyaki and Bánkúti Dent (Péterfy, 1912a).

Fleischmann (1913a, b; 1914; 1916; 1920; 1934; 1939a) invented a special half-seed quantity method when breeding the Rumai varieties. He divided the seed of the mother plants into two parts, one of which he reserved. The other half was sown in single-row, multi-location performance trials with three replications. The reserved seed of plants which did well in these trials was then multiplied, each in isolated plots (probably using hemp plants as a barrier). New mother plants were selected from these plots and the progeny of each was maintained and studied separately for further selection. In time there was a decrease in the number of families maintained from these mother plants, but even in the 1930s there were still records of a few families selected in 1908. In the late 1910s, Fleischmann allowed free pollination between the progeny of the same families, since the new mother plants were chosen from the performance trials, but the multiplication of elite lines was still done in isolation using the reserved seed. This method was, in practice, the application of the line cultivation method used for self-pollinating plants in an open-pollinated species. It is interesting to note that this strict inbreeding did not lead to a deterioration in the performance of the variety (Fleischmann, 1939a). A type of line cultivation similar to that used in wheat breeding was also employed by Teleki (1926) to breed the variety Szegedi Yellow Dent from Funk Yellow Dent.

Endre Pap, the breeder of the varieties Mindszentpusztai Yellow Dent and Mindszentpusztai White, used the widely known Ohio method for 15 generations in succession, which, in addition to fixing the variety traits and performance level, probably also led to inbreeding. When Pap observed that the yield increase of his varieties had reached a plateau, he changed to another breeding method (hybrid maize breeding).

At least 5000 fully-developed, tested lines were obtained from various open-pollinated populations and pedigrees in Martonvásár between 1953 and 1983. Only lines originating from the pedigrees: HMv 850, B 18/4, HMv 401, HMv 404-C, HMv 480 and HMv 403 were involved in seed production. (The other local lines listed in Table 6 were either of unknown origin or did not originate from Martonvásár.) From 1976 onwards more than 12,500 S_1–S_2 lines from nearly 120 populations were tested in the Martonvásár population improvement programme, but no commercial lines were produced. The reasons for this failure were summarised by Hadi (2003a, 2004) as follows: 1. The hybrid performance of the S_1 lines selected from the populations did not reach the level of that of the standard hybrids; 2. The initial stock was not genetically stable. Despite the fact that the first breeders were not aware of these criteria, long-term selection for performance and genetic stability starting from sources with high potential nevertheless led to the development of several varieties from which breeders were able to select outstanding lines in both Yugoslavia and Hungary.

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Short communication

INVESTIGATIONS ON THE PLANT STRUCTURE CHARACTERISTICS OF EMMER [*Triticum turgidum* ssp. *dicoccon* (Schrank)] LANDRACES UNDER INHOMOGENEOUS CONDITIONS

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As a consequence of the recent spread of organic crop production, there is an increasing demand on the market for foodstuffs and food raw materials of special quality. New interest is being evinced in old cereal species that have been ignored for a long period, such as einkorn and emmer. Their production is hindered, however, by the fact that no breeding has been carried out on these species for long decades, and the landraces currently available are not suited to modern cultivation conditions. The breeding of varieties with the required habit is hindered by the lack of information on the plant structure of the various landraces and on the environmental dependence and inheritance of the characters that determine plant structure. Earlier studies suggest that inhomogeneous environmental systems can be used to identify the temperature and light conditions under which the phenotypic differences responsible for plant structure are the greatest, thus allowing the inheritance of these traits to be investigated. When two emmer landraces originating from diverse climatic regions (MvGB 301 and MvGB 304) were grown in a gradient phytotron chamber, it was found that relatively higher temperatures were more suitable for pinpointing differences in plant height, as the difference between the two varieties decreased parallel to a drop in temperature. Within the temperature range investigated it is advisable to choose the taller variety as basic breeding stock for organic variety development, as its height is closer to the ideotype for organic varieties. The length of the last internode in MvGB 301 is independent of changes in temperature, indicating that the phenotype is stable for this trait. The results clearly demonstrate that it is possible to find types of emmer which are morphologically adapted to the requirements of organic farming and have a plant structure relatively little affected by the genotype × environment interaction.

Key words: emmer, *Triticum dicoccum* ssp. *dicoccon* Schrank, gradient plant growth chamber, plant height, organic production

Introduction

In recent years organic crop production has spread widely, both in the EU as a whole and in Hungary. This can be attributed primarily to changes in consumer habits and to the increasing demand for natural, healthy foodstuffs. There is an ever greater demand for foodstuffs and food raw materials with special quality and unusual flavours, which can be used to prepare novel dishes.

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Long-forgotten cereal species, such as einkorn and emmer, are thus re-appearing on the market. These species have numerous advantages over bread wheat under organic or "low input" growing conditions. It is predicted on the intensively developing organic market that emmer (*Triticum turgidum* ssp. *dicoccon*) will be the quickest cereal species to spread, as its quality, flavour and suitability for a wide range of uses have aroused customer interest in many countries. The rapid spread of emmer production is considerably hindered, however, since the species has not been bred in recent decades, and the landraces available in gene banks are not usually suited to modern production conditions. For use in organic farming, plants should be relatively tall (approx. 100 cm), the last internode should make up at least 50% of the stem, and the spike should have a loose structure. This type would ensure satisfactory lodging resistance and would protect the spike and grains from fungus infection, as they would be well above the leaf zone. The rapid development of this ideotype is complicated, however, by the lack of information on the plant structure of various landraces, on the extent to which this structure depends on the environment, and on the heritability of these traits. The results of earlier studies (Kőszegi and Kovács, 2004) suggest that experiments carried out in a gradient chamber providing an inhomogeneous environment make it possible to determine the temperature and light conditions under which the phenotypic differences between the genotypes are the greatest, thus accelerating the determination of the inheritance of these traits.

In the present studies the gradient chamber was used to determine the extent to which the plant height and the length of the last internode depended on changes in the temperature and light intensity in two emmer landraces which had proved to be of interest for breeding.

Materials and methods

The experiments were carried out on two winter emmer [*Triticum turgidum* ssp. *dicoccon* (Schrank) Thell.] landraces, with gene bank accession numbers MvGB 301 and MvGB 304, found to possess value for breeding. Mv GB 301 originated from the eastern slopes of the Ukrainian Carpathians, while MvGB 304 is found on the western slopes of the Caucasus. The experimental material was developed as described by Kőszegi and Kovács (2004), after which randomly selected grains, with the husks attached, were soaked in water at 20°C for 12 hours. After vernalisation for four weeks at 2°C, the seedlings were planted into pots, using the method generally employed for plant growth in the phytotron (Tischner et al., 1997). The pots were then placed in the gradient chamber in the Martonvásár phytotron, with the varieties in alternate rows (Tischner and Veisz, 1996). A temperature gradient of 8°C to 18°C was adjusted across the rows and a light intensity gradient of 210–540 μmol m^{-2} s^{-1} across the columns, with a daylength of 16 hours (Kőszegi et al., 2003).

Plant height and the length of the last internode were measured for each plant throughout the experiment. The results were evaluated using multifactorial statistical analysis (SPSS 10.0, 1993).

Results and discussion

The plant heights recorded for the two varieties at each temperature level are illustrated in Figure 1. The data obtained for the whole of the inhomogeneous system indicated that, of the two gradients, only the temperature had a significant influence on plant height. The values given on the x-axis represent low (1) to high (10) values of temperature and light intensity. It can be clearly seen, that as the temperature dropped there was a parallel rise in plant height, which could be described with a close to linear model. By contrast, although there were considerable deviations in the data, it appeared that the intensity of illumination had no consistent effect on plant height in the range studied. As these data clearly indicated that the plant height was not dependent on the light intensity, the effect of this gradient was not further analysed.

Substantial genotypic differences were observed between the two varieties with respect to plant height. It can be clearly seen on Figure 2 that the heights of the two landraces were significantly different, MvGB 304 growing significantly taller throughout the temperature range than MvGB 301 even under phytotron conditions. The figure also reveals that the two varieties responded quite differently to a reduction in temperature. The increase in plant height in MvGB 301 parallel to a decline in temperature could be adequately described by means of linear regression (R^2=0.94), while the linear model could only be accepted with reservations for MvGB 304 (R^2=0.68). The steepness of the regression line also revealed significant differences between the two varieties, indicating that MvGB 301 responded more sensitively to a decrease in temperature than MvGB 304.

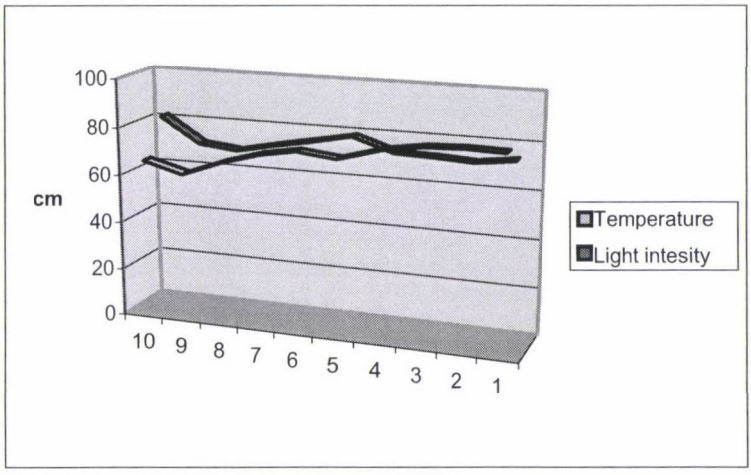

Fig. 1. Changes in mean plant height as a function of temperature and light gradients

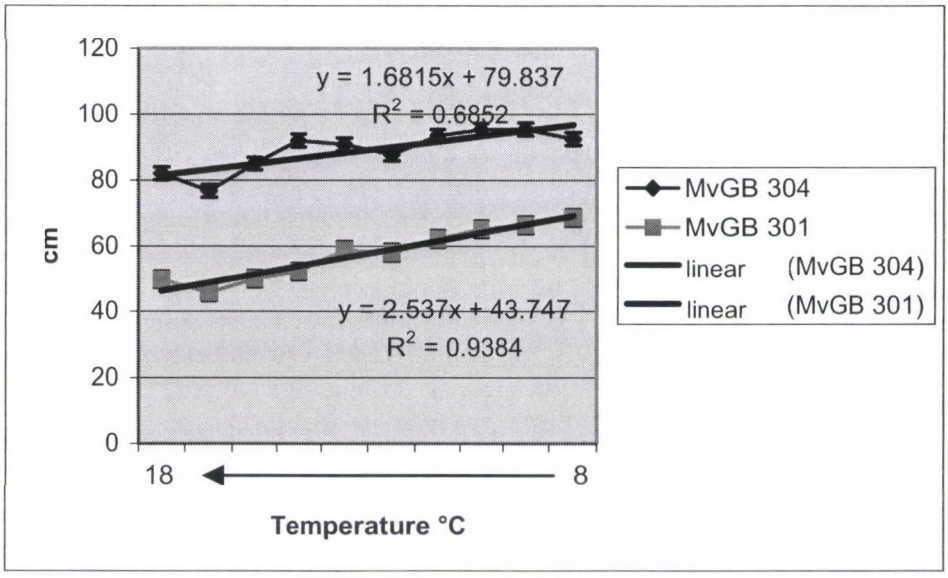

Fig. 2. Changes in the plant height of the two emmer genotypes as a function of temperature

The results suggest that relatively high temperatures are more suitable for identifying differences in plant height between the varieties, as these differences decreased as the temperature dropped, making it more difficult to detect the effect of minor modifying genes. Within the temperature range examined, the taller variety appears to be more suitable for use as basic breeding stock for developing organic varieties, since its height is closer to the ideotype for organic varieties.

As noted in the introduction, however, plant height is only one component in the desired phenotype. In organic farming it is advisable to grow plants where the spikes are at a considerable distance from the leaf zone, which can best be achieved by lengthening the last internode.

The results were somewhat surprising. The two varieties were quite different as regards the length of the last internode. In the case of MvGB 301 the last internode shortened as the temperature dropped (Fig. 3), although the opposite would have been expected from the increase in plant height (Fig. 2). By contrast, the length of the last internode in landrace MvGB 304 was practically constant, being independent of the decline in temperature. This means in practice that it retains its proportion of the whole plant height, ensuring that the spikes continue to grow well above the leaf zone. The results clearly indicate that it is possible to identify emmer genotypes morphologically suited to the requirements of organic production, whose plant structure is relatively little influenced by the genotype × environment interaction.

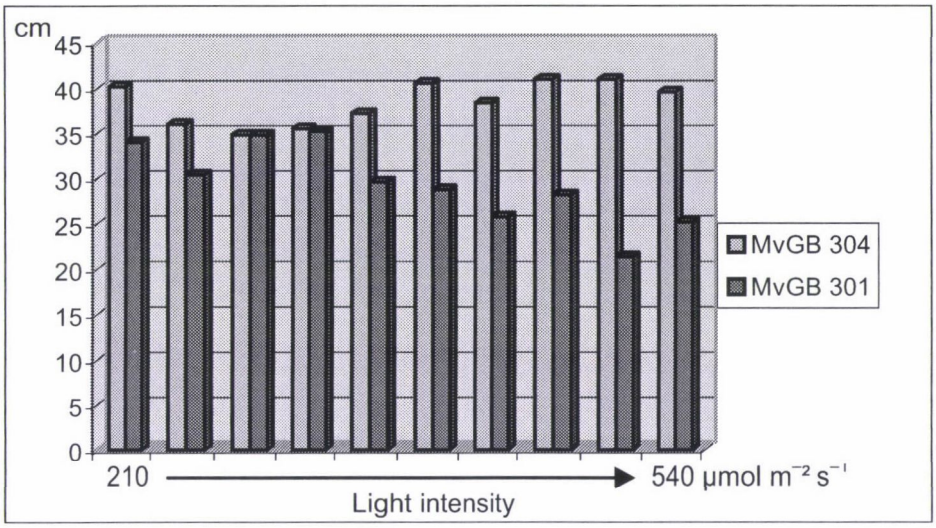

Fig. 3. Effect of the illumination intensity on the length of the last internode in the two emmer varieties

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